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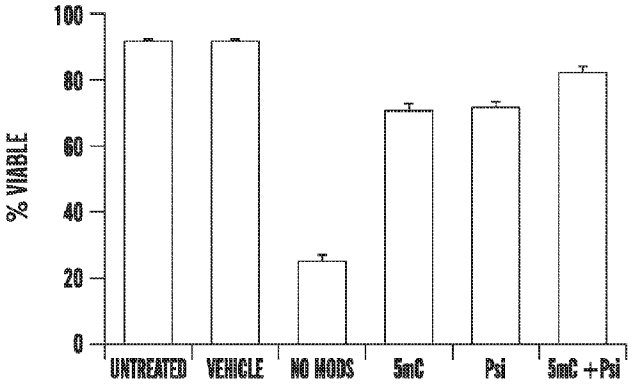

(57) Abrégé/Abstract:
Described herein are synthetic, modified RNAs for changing the phenotype of a cell, such as expressing a polypeptide or altering the developmental potential. Accordingly, provided herein are compositions, methods, and kits comprising synthetic, modified RNAs for changing the phenotype of a cell or cells. These methods, compositions, and kits comprising synthetic, modified RNAs can be used either to express a desired protein in a cell or tissue, or to change the differentiated phenotype of a cell to that of another, desired cell type.

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(54) Title: SUSTAINED POLYPEPTIDE EXPRESSION FROM SYNTHETIC, MODIFIED RNAS AND USES THEREOF

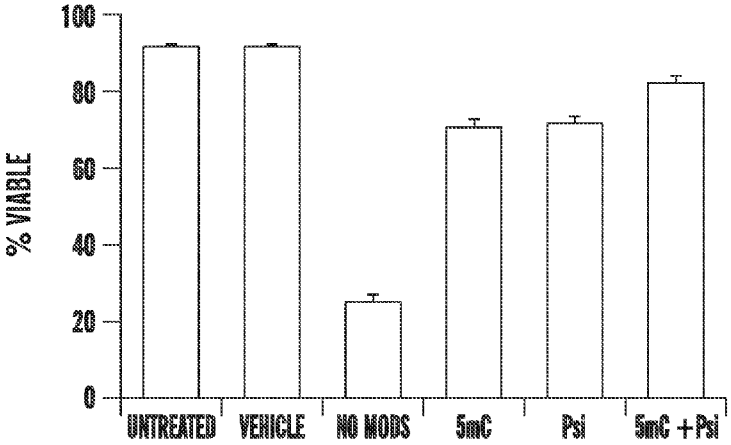

FIG. 2A

(57) Abstract: Described herein are synthetic, modified RNAs for changing the phenotype of a cell, such as expressing a polypeptide or altering the developmental potential. Accordingly, provided herein are compositions, methods, and kits comprising synthetic, modified RNAs for changing the phenotype of a cell or cells. These methods, compositions, and kits comprising synthetic, modified RNAs can be used either to express a desired protein in a cell or tissue, or to change the differentiated phenotype of a cell to that of another, desired cell type.

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SUSTAINED POLYPEPTIDE EXPRESSION FROM SYNTHETIC, MODIFIED RNAS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U.S.C. §119(e) of U.S. Provisional Patent Application Serial No.: 61/325,003 filed on April 16, 2010 and U.S. Provisional Patent Application Serial No.: 61/387,220 filed on September 28, 2010.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web. Said ASCII copy, created on April 8, 2011, is named 67442PCT.txt and is 7,196,077 bytes in size.

FIELD OF THE INVENTION

[0003] The field of the invention relates to synthetic, modified RNAs and uses thereof.

BACKGROUND

[0004] The ability to change the phenotype of a cell or cells, either to express a desired protein or to change the differentiated phenotype of the cell to that of another, desired cell type, has applications in both research and therapeutic settings. The phenotype of a cell is most commonly modified by expression of protein(s) from exogenous DNA or from recombinant viral vectors. These approaches have the potential for unintended mutagenic effects.

[0005] One area of interest is the modification of cellular differentiation such that cells are directed to different developmental lineages. As one example, generating insulin-producing pancreatic β cells from acinar pancreatic cells or other somatic cell types, has the potential to treat diabetes. As but one other example, the ability to redifferentiate a tumor cell or tumor stem cell to a non-cancerous cell type can provide a therapy for cancer. Current protocols for altering cell fate tend to focus on the expression of factors, such as differentiation factors, dedifferentiation factors, transdifferentiation factors, and reprogramming factors, using viral- or DNA-mediated expression.

[0006] An area of recent focus is the production of pluripotent or multipotent stem cells from non-embryonic sources. Induction of pluripotency was originally achieved by Yamanaka and colleagues using retroviral vectors to enforce expression of four transcription factors, KLF4, c-MYC, OCT4, and SOX2 (KMOS) (Takahashi, K. and S. Yamanaka, *Cell*, 2006. 126(4): p. 663-76 ; Takahashi, K., et al., *Cell,* 2007. 131(5): p. 861-72). Attempts to derive induced pluripotent stem (iPS) cells have also been made using excisable lentiviral and transposon vectors, or through repeated

application of transient plasmid, episomal, and adenovirus vectors (Chang, C.-W., et al., *Stem Cells*, 2009. 27(5): p. 1042-1049; Kaji, K., et al., *Nature*, 2009. 458(7239): p. 771-5; Okita, K., et al., *Science*, 2008. 322(5903): p. 949-53; Stadtfeld, M., et al., *Science*, 2008. 322(5903): p. 945-9; Woltjen, K., et al., *Nature*, 2009; Yu, J., et al., *Science*, 2009: p. 1172482; Fusaki, N., et al., *Proc Jpn Acad Ser B Phys Biol Sci*, 2009. 85(8): p. 348-62). Human pluripotent cells have also been derived using two DNA-free methods: serial protein transduction with recombinant proteins incorporating cell-penetrating peptide moieties (Kim, D., et al., *Cell Stem Cell*, 2009. 4(6): p. 472-476; Zhou, H., et al., *Cell Stem Cell*, 2009. 4(5): p. 381-4), and infectious transgene delivery using the Sendai virus, which has a completely RNA-based reproductive cycle (Fusaki, N., et al., *Proc Jpn Acad Ser B Phys Biol Sci*, 2009. 85(8): p. 348-62).

SUMMARY

[0007] Provided herein are compositions, methods, and kits for changing the phenotype of a cell or cells. These methods, compositions, and kits can be used either to express a desired protein in a cell or tissue, or to change the differentiated phenotype of a cell to that of another, desired cell type. Significantly, the methods, compositions, and kits described herein do not utilize exogenous DNA or viral vector-based methods for the expression of protein(s), and thus, do not cause permanent modification of the genome or have the potential for unintended mutagenic effects.

[0008] The compositions, methods, and kits described herein are based upon the direct introduction of synthetic RNAs into a cell, which, when translated, provide a desired protein or proteins. Higher eukaryotic cells have evolved cellular defenses against foreign, "non-self," RNA that ultimately result in the global inhibition of cellular protein synthesis, resulting in cellular toxicity. This response involves, in part, the production of Type I or Type II interferons, and is generally referred to as the "interferon response" or the "cellular innate immune response." The cellular defenses normally recognize synthetic RNAs as foreign, and induce this cellular innate immune response. The inventors have recognized that the ability to achieve sustained or repeated expression of an exogenously directed protein using synthetic RNA is hampered by the induction of this innate immune response. In the methods described herein, the effect of the cellular innate immune response is mitigated by using synthetic RNAs that are modified in a manner that avoids or reduces the response. Avoidance or reduction of the innate immune response permit sustained expression from exogenously introduced RNA necessary, for example, to modify the developmental phenotype of a cell. In one aspect, sustained expression is achieved by repeated introduction of synthetic, modified RNAs into a target cell or its progeny.

[0009] The modified, synthetic RNAs described herein, in one aspect, can be introduced to a cell in order to induce exogenous expression of a protein of interest in a cell. The ability to direct exogenous expression of a protein of interest using the modified, synthetic RNAs described herein is useful, for example, in the treatment of disorders caused by an endogenous genetic defect in a cell or

organism that impairs or prevents the ability of that cell or organism to produce the protein of interest. Accordingly, in some embodiments, compositions and methods comprising the modified, synthetic RNAs described herein can be used for the purposes of gene therapy.

[0010] The modified, synthetic RNAs described herein can advantageously be used in the alteration of cellular fates and/or developmental potential. The ability to express a protein from an exogenous RNA permits both the alteration or reversal of the developmental potential of a cell, *i.e.*, the reprogramming of the cell, and the directed differentiation of a cell to a more differentiated phenotype. A critical aspect in altering the developmental potential of a cell is the requirement for sustained and prolonged expression of one or more developmental potential altering factors in the cell or its immediate progeny. Traditionally, such sustained expression has been achieved by introducing DNA or viral vectors to a cell. These traditional approaches have limited therapeutic utility due to the potential for insertional mutagenesis. The compositions and methods described herein completely avoid such risks related to genomic alterations.

[0011] One of the areas that can most benefit from the ability to express a desired protein or proteins over a sustained period of time from exogenous synthetic, modified RNAs as described herein is the generation of pluripotent or multipotent cells from cells initially having a more differentiated phenotype. In this aspect, synthetic, modified RNAs encoding a reprogramming factor or factors are used to reprogram cells to a less differentiated phenotype, *i.e.*, having a greater developmental potential. Unexpectedly, the inventors have discovered that the synthetic, modified RNAs described herein permit both dramatically enhanced efficiency and rate of cellular reprogramming relative to DNA- or viral vector-mediated reprogramming methods.

[0012] A major goal of stem cell technology is to make the stem cell differentiate into a desired cell type, *i.e.*, directed differentiation. Not only are the compositions and methods described herein useful for reprogramming cells, they are also applicable to this directed differentiation of cells to a desired phenotype. That is, the same technology described herein for reprogramming is directly applicable to the differentiation of the reprogrammed cell, or any other stem cell or precursor cell, for that matter, to a desired cell type.

[0013] Accordingly, in one aspect, provided herein are synthetic, modified RNA molecules encoding a polypeptide, where the synthetic, modified RNA molecule comprises one or more modifications, such that introducing the synthetic, modified RNA molecule to a cell results in a reduced innate immune response relative to a cell contacted with a synthetic RNA molecule encoding the polypeptide not comprising the one or more modifications.

[0014] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule comprises at least two modified nucleosides. In one such embodiment, the two modified nucleosides are selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'deoxy uridine (2' dU), 4-thiouridine (s4U),

5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In one such embodiment of this aspect and all such aspects described herein, the at least two modified nucleosides are 5-methylcytidine (5mC) and pseudouridine.

[0015] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule further comprises a 5' cap. In one such embodiment, the 5' cap is a 5' cap analog. In one embodiment, the 5' cap analog is a 5' diguanosine cap.

[0016] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule does not comprise a 5' triphosphate.

[0017] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof. In one embodiment, the poly(A) tail, the Kozak sequence, the 3' untranslated region, the 5' untranslated region, or the any combination thereof comprises one or more modified nucleosides.

[0018] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule is further treated with an alkaline phosphatase.

[0019] In some embodiments of this aspect and all such aspects described herein, the innate immune response comprises expression of a Type I or Type II interferon.

[0020] In some embodiments of this aspect and all such aspects described herein, the innate immune response comprises expression of one or more IFN signature genes selected from the group consisting of IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20.

[0021] In another aspect, provided herein is a cell contacted with a synthetic, modified RNA molecule encoding a polypeptide, or a progeny cell of the contacted cell, where the synthetic, modified RNA molecule comprises one or more modifications, such that introducing the synthetic, modified RNA molecule to the cell results in a reduced innate immune response relative to the cell contacted with a synthetic RNA molecule encoding the polypeptide not comprising the one or more modifications.

[0022] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule contacted with the cell comprises at least two modified nucleosides. In one such embodiment, the two modified nucleosides are selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-

trimethylguanosine (m2,2,7G), and inosine (I). In one such embodiment of this aspect and all such aspects described herein, the at least two modified nucleosides are 5-methylcytidine (5mC) and pseudouridine.

[0023] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule contacted with the cell further comprises a 5' cap. In one such embodiment, the 5' cap is a 5' cap analog. In one embodiment, the 5' cap analog is a 5' diguanosine cap.

[0024] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule contacted with the cell does not comprise a 5' triphosphate.

[0025] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule contacted with the cell further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof. In one embodiment, the poly(A) tail, the Kozak sequence, the 3' untranslated region, the 5' untranslated region, or the any combination thereof comprises one or more modified nucleosides.

[0026] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule contacted with the cell is further treated with an alkaline phosphatase.

[0027] In some embodiments of this aspect and all such aspects described herein, the innate immune response comprises expression of a Type I or Type II interferon, and the expression of the Type I or Type II interferon is not increased more than three-fold compared to a reference from a cell which has not been contacted with the synthetic modified RNA molecule.

[0028] In some embodiments of this aspect and all such aspects described herein, the innate immune response comprises expression of one or more IFN signature genes selected from the group consisting of IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20, and where the expression of the one ot more IFN signature genes is not increased more than six-fold compared to a reference from a cell which has not been contacted with the synthetic modified RNA molecule.

[0029] In some embodiments of this aspect and all such aspects described herein, the polypeptide encoded by the synthetic, modified RNA molecule introduced to the cell alters a function or a developmental phenotype of the cell. In some such embodiments, the developmental phenotype is a developmental potential. In some embodiments, the developmental potential is decreased. In some embodiments, the developmental potential is increased.

[0030] In some embodiments of this aspect and all such aspects described herein, the polypeptide encoded by the synthetic, modified RNA molecule is a reprogramming factor, a differentiation factor, or a de-differentiation factor.

[0031] In another aspect, provided herein is a cell contacted with a synthetic, modified RNA molecule encoding a polypeptide, or a progeny cell of the contacted cell, where expression of the

encoded polypeptide in the cell alters a function or a developmental phenotype of the cell, and where the synthetic, modified RNA molecule comprises one or more modifications, such that introducing the synthetic, modified RNA molecule to the cell results in a reduced innate immune response relative to the cell contacted with a synthetic RNA molecule encoding the polypeptide not comprising the one or more modifications.

[0032] In some embodiments of this aspect and all such aspects described herein, the developmental phenotype altered by expression of the polypeptide encoded by the synthetic, modified RNA molecule is a developmental potential. In some such embodiments of this aspect, the developmental potential is decreased. In other such embodiments of this aspect, the developmental potential is increased.

[0033] In some embodiments of these aspects and all such aspects described herein, the polypeptide encoded by the synthetic, modified RNA molecule is a reprogramming factor, a differentiation factor, or a de-differentiation factor.

[0034] In another aspect, provided herein is a pluripotent cell, where the pluripotent cell is not an embryonic stem cell, and where the cell was not induced by viral expression of one or more reprogramming factors, and where the cell, when subjected to an unsupervised hierarchical cluster analysis, clusters more closely to an embryonic stem cell than does a pluripotent cell induced by viral expression of one or more reprogramming factors, exogenous protein introduction of one or more reprogramming factors, small molecule mediated expression or induction of one or more reprogramming factors, or any combination thereof.

[0035] In one such aspect, provided herein is pluripotent cell, where the pluripotent cell is not an embryonic stem cell, and where the cell was not induced by viral expression of one or more reprogramming factors, and where the cell subjected to an unsupervised hierarchical cluster analysis clusters more closely to a human embryonic stem cell than does a pluripotent cell induced by viral expression of one or more reprogramming factors.

[0036] In some embodiments of these aspects and all such aspects described herein, the unsupervised hierarchical cluster analysis is performed on the pluripotent cells using a Euclidean distance with average linkage method, in which the similarity metric for comparison between different cells is indicated on the height of cluster dendrogram.

[0037] In some embodiments of these aspects and all such aspects described herein, the unsupervised hierarchical cluster analysis is performed on the pluripotent cells using a data set selected from the group consisting of gene expression data, protein expression data, DNA methylation data, histone modification data, and microRNA data.

[0038] In some embodiments of these aspects and all such aspects described herein, the pluripotent cell is generated from a precursor somatic cell contacted with at least one synthetic, modified RNA encoding a reprogramming factor.

[0039] In some embodiments of these aspects and all such aspects described herein, the pluripotent cell is generated from a precursor human somatic cell.

[0040] Another aspect provides a cell comprising an exogenously introduced modified, synthetic RNA encoding a developmental potential altering factor.

[0041] In some embodiments of this aspect and all such aspects described herein, the cell is a human cell. In other embodiments of this aspect and all such aspects described herein, the cell is not a human cell.

[0042] In some embodiments of this aspect and all such aspects described herein, the cell or its immediate precursor cell(s) has been subjected to at least 3 separate rounds of contacting with the exogenously introduced modified synthetic RNA encoding the developmental potential altering factor.

[0043] In some embodiments of this aspect and all such aspects described herein, the cell has a reduced expression of a Type I or Type II IFN relative to a cell subjected to at least 3 separate rounds of contacting with an exogenously introduced non-modified, synthetic RNA encoding the developmental potential altering factor.

[0044] In some embodiments of this aspect and all such aspects described herein, the cell has a reduced expression of at least one IFN-signature gene relative to a cell subjected to at least 3 separate rounds of contacting with an exogenously introduced non-modified synthetic RNA encoding the developmental potential altering factor.

[0045] In one such embodiment of this aspect and all such aspects described herein, the IFN-signature gene is selected from the group consisting of IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20.

[0046] In some embodiments of this aspect and all such aspects described herein, the developmental potential altering factor is a reprogramming factor, a differentiation factor, or a de-differentiation factor.

[0047] In one such embodiment of this aspect and all such aspects described herein, the reprogramming factor is selected from the group consisting of: OCT4 (SEQ ID NO: 788), SOX1, SOX 2 (SEQ ID NO: 941 or SEQ ID NO: 1501), SOX 3, SOX15, SOX 18, NANOG, KLF1, KLF 2, KLF 4 (SEQ ID NO: 501), KLF 5, NR5A2, c-MYC (SEQ ID NO: 636), l- MYC, n- MYC, REM2, TERT, and LIN28 (SEQ ID NO: 524). In some embodiments of this aspect and all such aspects described herein, the reprogramming factor is not c-MYC.

[0048] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor comprises at least two modified nucleosides. In one such embodiment, the two modified nucleosides are selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine

(m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In one such embodiment of this aspect and all such aspects described herein, the at least two modified nucleosides are 5-methylcytidine (5mC) and pseudouridine.

[0049] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor further comprises a 5' cap. In one such embodiment, the 5' cap is a 5' cap analog. In one embodiment, the 5' cap analog is a 5' diguanosine cap.

[0050] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor does not comprise a 5' triphosphate.

[0051] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof. In one embodiment, the poly(A) tail, the Kozak sequence, the 3' untranslated region, the 5' untranslated region, or the any combination thereof comprises one or more modified nucleosides.

[0052] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor is further treated with an alkaline phosphatase.

[0053] In some embodiments of this aspect and all such aspects described herein, the cell or its immediate precursor cell(s) is derived from a somatic cell, a partially reprogrammed somatic cell, a pluripotent cell, a multipotent cell, a differentiated cell, or an embryonic cell.

[0054] In another aspect, provided herein is a composition comprising at least one modified, synthetic RNA encoding a reprogramming factor, and cell growth media.

[0055] In some embodiments of this aspect and all such aspects described herein, the composition permits an efficiency of pluripotent cell generation from a starting population of somatic cells of at least 1%.

[0056] In some embodiments of this aspect and all such aspects described herein, the composition permits a rate of pluripotent cell generation from a starting population of somatic cells of less than 25 days and greater than 7 days.

[0057] In one embodiment of this aspect and all such aspects described herein, the reprogramming factor is selected from the group consisting of: OCT4, SOX1, SOX 2, SOX 3, SOX15, SOX 18, NANOG, KLF1, KLF 2, KLF 4, KLF 5, NR5A2, c-MYC, l- MYC, n- MYC, REM2, TERT, and LIN28.

[0058] In some embodiments of this aspect and all such aspects described herein, the composition comprises at least 3 synthetic, modified, RNAs encoding at least 3 different reprogramming factors. In one such embodiment, the at least 3 different reprogramming factors encoded by the at least 3 synthetic, modified RNAs are selected from the group consisting of OCT4, SOX2, KLF4, c-MYC, and LIN-28.

[0059] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor comprises at least two modified nucleosides. In one such embodiment, the two modified nucleosides are selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In one such embodiment of this aspect and all such aspects described herein, the at least two modified nucleosides are 5-methylcytidine (5mC) and pseudouridine.

[0060] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor further comprises a 5' cap. In one such embodiment, the 5' cap is a 5' cap analog. In one embodiment, the 5' cap analog is a 5' diguanosine cap.

[0061] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor does not comprise a 5' triphosphate.

[0062] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof. In one embodiment, the poly(A) tail, the Kozak sequence, the 3' untranslated region, the 5' untranslated region, or the any combination thereof comprises one or more modified nucleosides.

[0063] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor is further treated with an alkaline phosphatase.

[0064] Another aspect provides a pluripotent cell generated using any of the compositions described herein.

[0065] In one aspect, provided herein is a cell composition comprising a pluripotent cell clone isolated from a population of somatic cells contacted a plurality of times with at least one synthetic, modified RNA encoding a developmental potential altering factor.

[0066] In some embodiments of this aspect and all such aspects described herein, the population of somatic cells is a population of human somatic cells.

[0067] In some embodiments of this aspect and all such aspects described herein, the pluripotent cell clone subjected to an unsupervised hierarchical cluster analysis clusters more closely to a human embryonic stem cell than does a pluripotent cell clone induced by viral expression of one or more reprogramming factors, exogenous protein introduction of one or more reprogramming factors, small molecule mediated expression or induction of one or more reprogramming factors, or any combination thereof.

[0068] Provided herein are methods of altering the developmental potential of a cell. In one aspect, the method comprises contacting with or introducing to a cell population or progeny cells thereof at least one synthetic, modified RNA encoding a developmental potential altering factor. In some embodiments of this aspect and all such aspects described herein, the contacting with or introducing to is performed at least three times.

[0069] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor comprises at least two modified nucleosides. In one such embodiment, the two modified nucleosides are selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In one such embodiment of this aspect and all such aspects described herein, the at least two modified nucleosides are 5-methylcytidine (5mC) and pseudouridine.

[0070] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor further comprises a 5' cap. In one such embodiment, the 5' cap is a 5' cap analog. In one embodiment, the 5' cap analog is a 5' diguanosine cap.

[0071] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor does not comprise a 5' triphosphate.

[0072] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof. In one embodiment, the poly(A) tail, the Kozak sequence, the 3' untranslated region, the 5' untranslated region, or the any combination thereof comprises one or more modified nucleosides.

[0073] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the developmental potential altering factor is further treated with an alkaline phosphatase.

[0074] In some embodiments of this aspect and all such aspects described herein, the method further comprises a step of determining that the cell population or progeny cells thereof maintain increased viability by measuring viability of the cell population or progeny cells thereof, where the viability of at least 50% of the contacted cell population or progeny cells thereof indicates that the cells maintain increased viability.

[0075] In some embodiments of this aspect and all such aspects described herein, the method further comprises a step of determining that the cell population or progeny cells thereof does not have a significant increase in expression of a Type I or a Type II IFN by measuring expression of a Type I or a Type II IFN in the contacted cell population or progeny cells thereof, where a less than three-fold increase in expression of Type I or Type II IFN in the contacted cell population or progeny cells thereof compared to cells that have not been contacted with the synthetic and modified RNA indicates that the cell population does not have a significant increase in expression of Type I or Type II IFN.

[0076] In some such embodiments of this aspect and all such aspects described herein, measuring the expression of Type I or Type II IFN is performed by measuring expression of at least one IFN-signature gene selected from IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20, where a less than six-fold increase in expression of the at least one IFN-signature gene compared to the cell population or progeny cells thereof prior to contacting the cell population or progeny cells thereof with the at least one modified and synthetic RNA.

[0077] In some embodiments of this aspect and all such aspects described herein, contacting of the cell population or progeny cells thereof is performed *in vitro*, *ex vivo*, or *in vivo*.

[0078] Also provided herein are methods for reprogramming a somatic cell into a pluripotent cell. In one aspect, the method comprises contacting a somatic cell population or progeny cells thereof with at least one modified, synthetic RNA encoding at least one reprogramming factor at least five consecutive times.

[0079] In some embodiments of this aspect and all such aspects described herein, the at least five consecutive times occur within 25 days.

[0080] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic, modified RNA encoding the reprogramming factor comprises at least two modified nucleosides. In one such embodiment, the at least two modified nucleosides are selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-

methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In one such embodiment, the at least two modified nucleosides are 5-methylcytidine (5mC) and pseudouridine.

[0081] In one such embodiment of this aspect and all such aspects described herein, the at least two modified nucleosides are 5-methylcytidine (5mC) and pseudouridine.

[0082] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the reprogramming factor further comprises a 5' cap. In one such embodiment, the 5' cap is a 5' cap analog. In one embodiment, the 5' cap analog is a 5' diguanosine cap.

[0083] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the reprogramming factor does not comprise a 5' triphosphate.

[0084] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding the reprogramming factor further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof. In one embodiment, the poly(A) tail, the Kozak sequence, the 3' untranslated region, the 5' untranslated region, or the any combination thereof comprises one or more modified nucleosides.

[0085] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA molecule encoding th reprogramming factor is further treated with an alkaline phosphatase.

[0086] In some embodiments of this aspect and all such aspects described herein, the at least one reprogramming factor is selected from: OCT4 (SEQ ID NO: 788), SOX1, SOX 2 (SEQ ID NO: 941 or SEQ ID NO: 1501), SOX 3, SOX15, SOX 18, NANOG, KLF1, KLF 2, KLF 4 (SEQ ID NO: 501), KLF 5, NR5A2, c-MYC (SEQ ID NO: 636), l- MYC, n- MYC, REM2, TERT, and LIN28 (SEQ ID NO: 524). In some embodiments of this aspect and all such aspects described herein, the reprogramming factor is not c-MYC.

[0087] In some embodiments of this aspect and all such aspects described herein, the at least one reprogramming factor comprises a synthetic and modified RNA encoding OCT4, a synthetic and modified RNA encoding SOX2, a synthetic and modified RNA encoding c-MYC, and a synthetic and modified RNA encoding KLF4. In some embodiments of this aspect and all such aspects described herein, the at least one reprogramming factor further comprises a synthetic and modified RNA molecule encoding LIN28.

[0088] In some embodiments of this aspect and all such aspects described herein, a combination of at least three reprogramming selected from the group consisting of a synthetic, modified RNA encoding OCT4, a synthetic, modified RNA encoding SOX2, a synthetic, modified RNA encoding c-MYC, a synthetic, modified RNA encoding KLF4, and a synthetic, modified RNA molecule encoding LIN28, are used in the methods described herein.

[0089] In some embodiments of this aspect and all such aspects described herein, the method further comprises determining increased reprogramming efficiency of the somatic cell by measuring efficiency of reprogramming, where efficiency of at least 1% is indicative of increased reprogramming efficiency.

[0090] In some embodiments of this aspect and all such aspects described herein, the method further comprises a step of determining that the somatic cell or progeny cells thereof maintain increased viability by measuring viability of the somatic cell or progeny cells thereof, where viability of at least 50% of the contacted somatic cell or progeny cells thereof indicates that the cells maintain increased viability.

[0091] In some embodiments of this aspect and all such aspects described herein, the method further comprises the step of determining that the reprogrammed somatic cell produced by the method has an increased likeness to the potency of an embryonic stem cell by subjecting the pluripotent cell or pluripotent cell population generated by the method to an unsupervised hierarchical cluster analysis and comparing it to a reference from an unsupervised cluster analysis of a pluripotent cell produced by viral expression of one or more of the reprogramming factors, exogenous protein introduction of one or more reprogramming factors, small molecule mediated expression or induction of one or more reprogramming factors, such that if the reprogrammed somatic cell clusters more closely to an embryonic stem cell than it does to a the reference, it has an increased likeness to the potency of embryonic stem cell.

[0092] In some embodiments of this aspect and all such aspects described herein, the method further comprises a step of determining that the reprogrammed somatic cell or progeny cell thereof does not have a significant increase in expression of IFN by measuring expression of at least one IFN-signature gene in the reprogrammed somatic cell or progeny cell thereof, such that if the increase in expression of the at least one IFN-signature gene is less than six-fold compared to a reference from a somatic cell prior to it being subjected to reprogramming indicates that the reprogrammed somatic cell or progeny cell thereof does not have a significant increase in expression of IFN.

[0093] In some such embodiments of this aspect and all such aspects described herein, the method further comprises the IFN-signature gene is selected from the group consisting of IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20.

[0094] In some embodiments of this aspect and all such aspects described herein, the somatic cell population or progeny cells thereof are contacted under a low-oxygen condition.

[0095] In some embodiments of this aspect and all such aspects described herein, the method further comprises determining that the reprogrammed somatic cell or progeny thereof expresses sufficient levels of genes to determine pluripotency by measuring expression of at least two genes selected from the group consisting of SOX2, REX1, DNMT3B, TRA-1-60, TRA-1-81, SSEA3,

SSEA4, OCT4, and NANOG and comparing the result to a reference from an embryonic stem cell, such that if at least two of the genes are expressed at the level they are expressed in the embryonic stem cell, it indicates that the reprogrammed somatic cell or progeny thereof expresses sufficient levels of genes to determine pluripotency.

[0096] In some embodiments of this aspect and all such aspects described herein, contacting of the somatic cell population or progeny cells thereof is performed *in vitro*, *ex vivo*, or *in vivo*.

[0097] In some embodiments of this aspect and all such aspects described herein, the somatic cell is a human somatic cell.

[0098] Other aspects described herein provide methods of treating subjects in need of cellular therapies. In such aspects, an effective amount of a population of any of the progenitor, multipotent, oligopotent, lineage-restricted, fully or partially differentiated cells, generated using any of the compositions or methods comprising synthetic, modified RNAs described herein, is administered to a subject in need of a cellular therapy. Also provided herein are methods of treating subjects in need of treatment for a disease or disorder by administering any of the pharmaceutical compositions comprising synthetic, modified RNAs described herein.

[0099] Accordingly, in one aspect, provided herein is a method of treating a subject in need of a cellular therapy, comprising: administering to a subject in need of a cellular therapy an effective amount of a population of cells having altered developmental potential produced by contacting a cell population or progeny cells thereof with at least one synthetic, modified RNA encoding a developmental potential altering factor for at least three consecutive times.

[00100] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic and modified RNA encoding a developmental potential altering factor comprises at least two modified nucleosides. In one embodiment of this aspect, the at least two modified nucleosides are selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In one embodiment of this aspect, the at least two modified nucleosides are 5-methylcytidine (5mC) and pseudouridine.

[00101] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic and modified RNA encoding a developmental potential altering factor at least one synthetic, modified RNA further comprises a 5' cap. In one embodiment of this aspect, the 5' cap is a 5' cap analog. In one such embodiment, the 5' cap analog is a 5' diguanosine cap.

[00102] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic and modified RNA encoding a developmental potential altering factordoes not comprise a 5' triphosphate.

[00103] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic, modified RNA encoding a developmental potential altering factor further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof.

[00104] In some embodiments of this aspect and all such aspects described herein, the contacting at least three consecutive times are at least 24 hours apart. In some embodiments of this aspect and all such aspects described herein, the contacting at least three consecutive times occur within 15 days.

[00105] In some embodiments of this aspect and all such aspects described herein, the method further comprises a step of obtaining an autologous cell from the subject and generating a population of cells having altered developmental potential from the autologous cell by contacting the cell population or progeny cells thereof with at least one synthetic, modified RNA encoding a developmental potential altering factor for at least three consecutive times.

[00106] In some embodiments of this aspect and all such aspects described herein, the method further comprises a step of determining that the population of cells having altered developmental potential does not have a significant increase in expression of Type I or Type II IFN prior to administering the population of cells having altered developmental potential to the subject, the step comprising measuring expression of Type I or Type II IFN, where expression that is less than three-fold compared to a reference from a cell that has not been subject to a treatment to alter developmental potential indicates that the population of cells having altered developmental potential does not have a significant increase in expression of Type I or Type II IFN.

[00107] In some such embodiments of this aspect and all such aspects described herein, the expression of Type I or Type II IFN expression is measured by measuring expression of at least one IFN-signature gene selected from the group consisting of IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20, and where increase of less than six-fold of the at least two IFN-signature genes indicates that the population of cells having altered developmental potential does not have a significant increase in expression of Type I or Type II IFN.

[00108] In some embodiments of this aspect and all such aspects described herein, the altered developmental potential is pluripotency.

[00109] In some such embodiments of this aspect and all such aspects described herein, the developmental potential altering factor is a reprogramming factor selected from the group consisting of: OCT4 (SEQ ID NO: 788), SOX1, SOX 2 (SEQ ID NO: 941 or SEQ ID NO: 1501), SOX 3, SOX15, SOX 18, NANOG, KLF1, KLF 2, KLF 4 (SEQ ID NO: 501), KLF 5, NR5A2, c-MYC (SEQ

ID NO: 636), l- MYC, n- MYC, REM2, TERT, and LIN28 (SEQ ID NO: 524). In some embodiments of this aspect and all such aspects described herein, the reprogramming factor is not c-MYC.

[00110] In some embodiments of this aspect and all such aspects described herein, the population of cells having altered developmental potential is of a lineage selected from one of an ecotodermal lineage, a mesodermal lineage, or an endodermal lineage.

[00111] In some embodiments of this aspect and all such aspects described herein, the population of cells having altered developmental potential is multipotent. In some embodiments of this aspect and all such aspects described herein, the population of cells having altered developmental potential is oligopotent. In some embodiments of this aspect and all such aspects described herein, the population of cells being administered is partially or fully differentiated.

[00112] In some embodiments of this aspect and all such aspects described herein, the population of cells having altered developmental potential is differentiated into at least one differentiated cell population.

[00113] Other aspects described herein provide compositions comprising synthetic, modified RNAs described herein and any of the progenitor, multipotent, oligopotent, lineage-restricted, fully or partially differentiated cells generated using any of the compositions or methods described herein for use in treating subjects in need of treatment for a disease or disorder or in need of cellular therapies. In such aspects, an effective amount of a population of any of the progenitor, multipotent, oligopotent, lineage-restricted, fully or partially differentiated cells, generated using any of the compositions or methods comprising synthetic, modified RNAs described herein,can be administered to a subject in need of a cellular therapy.

[00114] Accordingly, in one aspect, provided herein is a population of cells having altered developmental potential for use in treating a subject in need of a cellular therapy, where the population of cells having altered developmental potential is produced by contacting a cell population or progeny cells thereof with at least one synthetic, modified RNA encoding a developmental potential altering factor. In some embodiments of this aspect and all such aspects described herein, contacting occurs for at least three consecutive times.

[00115] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic and modified RNA comprises at least two modified nucleosides. In one such embodiment of this aspect and all such aspects described herein, the at least two modified nucleosides are selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In one such

embodiment of this aspect and all such aspects described herein, the at least two modified nucleosides are 5-methylcytidine (5mC) and pseudouridine.

[00116] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic, modified RNA further comprises a 5' cap. In one embodiment of this aspect and all such aspects described herein, the 5' cap is a 5' cap analog. In one such embodiment,the 5' cap analog is a 5' diguanosine cap.

[00117] In some embodiments of this aspect and all such aspects described herein,the at least one synthetic, modified RNA does not comprise a 5' triphosphate.

[00118] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic, modified RNA further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof.

[00119] In some embodiments of this aspect and all such aspects described herein, the at least three consecutive times are at least 24 hours apart. In some embodiments of this aspect and all such aspects described herein, the at least three consecutive times occur within 15 days.

[00120] In some embodiments of this aspect and all such aspects described herein, the use further comprises obtaining an autologous cell from the subject and generating the population of cells having altered developmental potential from the autologous cell by contacting the cell population or progeny cells thereof with at least one synthetic, modified RNA encoding a developmental potential altering factor. In some embodiments of this aspect and all such aspects described herein, the contacting is for at least three consecutive times.

[00121] In some embodiments of this aspect and all such aspects described herein, the use further comprises determining that the population of cells having altered developmental potential does not have a significant increase in expression of Type I or Type II IFN prior to using the population of cells having altered developmental potential in the subject in need of cellular therapy, the determining comprising measuring expression of Type I or Type II IFN, wherein expression that is less than three-fold compared to a reference from a cell that does not have altered developmental potential indicates that the population of cells having altered developmental potential does not have a significant increase in expression of Type I or Type II IFN.

[00122] In some such embodiments of this aspect and all such aspects described herein, the expression of Type I or Type II IFN expression is measured by measuring expression of at least one IFN-signature gene selected from the group consisting of IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20, and wherein increase of less than six-fold of the at least two IFN-signature genes indicates that the population of cells having altered developmental potential does not have a significant increase in expression of Type I or Type II IFN.

[00123] In some embodiments of this aspect and all such aspects described herein, the altered developmental potential is pluripotency.

[00124] In some embodiments of this aspect and all such aspects described herein, the developmental potential altering factor is a reprogramming factor selected from the group consisting of: OCT4 (SEQ ID NO: 788), SOX1, SOX 2 (SEQ ID NO: 941 or SEQ ID NO: 1501), SOX 3, SOX15, SOX 18, NANOG, KLF1, KLF 2, KLF 4 (SEQ ID NO: 501), KLF 5, NR5A2, c-MYC (SEQ ID NO: 636), l- MYC, n- MYC, REM2, TERT, and LIN28 (SEQ ID NO: 524). In some embodiments of this aspect and all such aspects described herein, the reprogramming factor is not c-MYC.

[00125] In some embodiments of this aspect and all such aspects described herein, the population of cells having altered developmental potential is of a lineage selected from one of an ecotodermal lineage, a mesodermal lineage, or an endodermal lineage.

[00126] In some embodiments of this aspect and all such aspects described herein, the population of cells having altered developmental potential is multipotent.

[00127] In some embodiments of this aspect and all such aspects described herein,the population of cells having altered developmental potential is differentiated into at least one differentiated cell population.

[00128] Also provided herein are methods for identifying agents that have effects on a cellular phenotype or cellular parameter. In some aspects, provided herein are methods for identifying an agent that has an effect on a cellular phenotype. In one aspect, the method comprises: (a) contacting a cell with a synthetic, modified RNA encoding a polypeptide in an amount and frequency sufficient to alter the phenotype of the cell to that of a desired phenotype; (b) contacting the altered cell with a candidate agent; (c) assaying the desired phenotype in the presence of the candidate agent, where a change in the phenotype in the presence of the candidate agent indicates the agent has an effect on the phenotype.

[00129] In some embodiments of this aspect and all such aspects described herein, the polypeptide encoded by the synthetic, modified RNA is a reprogramming factor. In some embodiments of this aspect and all such aspects described herein, the polypeptide encoded by the synthetic, modified RNA is a differentiating factor.

[00130] In some embodiments of this aspect and all such aspects described herein,the cell is a pluripotent or multipotent cell.

[00131] In some embodiments of this aspect and all such aspects described herein, the cellular phenotype is viability, cell growth, expression of a cell-surface marker, or a functional parameter. In some such embodiments of this aspect and all such aspects described herein, the functional parameter is an electrophysiological parameter, an immunological parameter, or a metabolic parameter. In some embodiments, the metabolic parameter is insulin synthesis or insulin secretion. In some embodiments, the electrophysiological parameter is contractibility.

[00132] Also provided herein are kits for altering the phenotype or developmental potential of a cell. In one aspect, provided herein is a kit comprising: a) a container with at least one synthetic,

modified RNA molecule comprising at least two modified nucleosides, and b) packaging and instructions therefor.

[00133] In some embodiments of this aspect and all such aspects described herein, the kit further comprises a container with cell culture medium.

[00134] In some embodiments of this aspect and all such aspects described herein, the kit further comprises an IFN inhibitor. In some embodiments of this aspect and all such aspects described herein, the kit further comprises valproic acid.

[00135] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic, modified RNA encodes a developmental potential altering factor.

[00136] In some embodiments of this aspect and all such aspects described herein, the developmental potential altering factor is a reprogramming factor, a differentiation factor, or a de-differentiation factor.

[00137] In some embodiments of this aspect and all such aspects described herein, the synthetic, modified RNA encoding a reprogramming factor in the container has a concentration of 100 ng/µl. In some such embodiments of this aspect and all such aspects described herein, the reprogramming factor is selected from the group consisting of OCT4 (SEQ ID NO: 788), SOX1, SOX 2 (SEQ ID NO: 941 or SEQ ID NO: 1501), SOX 3, SOX15, SOX 18, NANOG, KLF1, KLF 2, KLF 4 (SEQ ID NO: 501), KLF 5, NR5A2, c-MYC (SEQ ID NO: 636), l- MYC, n- MYC, REM2, TERT, and LIN28 (SEQ ID NO: 524). In some such embodiments of this aspect and all such aspects described herein, the kit comprises at least three of the reprogramming factors. In some such embodiments of this aspect and all such aspects described herein, the at least three reprogramming factors comprise a synthetic, modified RNA encoding OCT4, a synthetic, modified RNA encoding SOX2, a synthetic, modified RNA encoding c-MYC, and a synthetic, modified RNA encoding KLF4. In some such embodiments of this aspect and all such aspects described herein, the total concentration of the reprogramming factors in the container is 100 ng/µl, and OCT4 is provided in molar excess of about three times the concentration of KLF4, SOX-2, and c-MYC. In some such embodiments of this aspect and all such aspects described herein, the kit further comprises a synthetic, modified RNA molecule encoding LIN28.

[00138] In some embodiments of this aspect and all such aspects described herein, the kit does not comprise a synthetic, modified RNA encoding c-MYC.

[00139] In some embodiments of this aspect and all such aspects described herein, the at least two modified nucleosides of the synthetic, modified RNA are selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine

(m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In some embodiments of this aspect and all such aspects described herein, the at least two modified nucleosides are 5-methylcytidine (5mC) and pseudouridine.

[00140] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic, modified RNA further comprises a 5' cap. In some such embodiments of this aspect and all such aspects described herein, the 5' cap is a 5' cap analog. In one embodiment of this aspect and all such aspects described herein, the 5' cap analog is a 5' diguanosine cap.

[00141] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic, modified RNA does not comprise a 5' triphosphate.

[00142] In some embodiments of this aspect and all such aspects described herein, the at least one synthetic and modified RNA further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated regions, or any combination thereof. In some such embodiments of this aspect and all such aspects described herein, the poly(A) tail, the Kozak sequence, the 3' untranslated region, the 5' untranslated region, or the any combination thereof, comprises one or more modified nucleosides.

[00143] In some embodiments of this aspect and all such aspects described herein, the kit further comprises a non-implantable delivery device or an implantable delivery device to deliver the at least one synthetic, modified RNA. In some such embodiments of this aspect and all such aspects described herein, the non-implantable delivery device is a pen device. In some such embodiments, the implantable delivery device is a pump, semi-permanent stent, or reservoir.

[00144] Another aspect provides a kit for reprogramming a somatic cell to an induced pluripotent stem cell, the kit comprising: a) a vial comprising a synthetic, modified RNA encoding an OCT4 reprogramming factor and a buffer; b) a vial comprising a synthetic, modified RNA encoding a SOX2 reprogramming factor and a buffer; c) a vial comprising a synthetic, modified RNA encoding a c-MYC reprogramming factor and a buffer; d) a vial comprising a synthetic, modified RNA encoding a KLF4 reprogramming factor and a buffer; and e) packaging and instructions therefor; where each of the synthetic, modified RNAs encoding a reprogramming factor comprise at least two modified nucleosides.

[00145] In some embodiments of this aspect and all such aspects described herein, the at least two modified nucleosides are pseudouridine and 5-methylcytodine.

[00146] In some embodiments of this aspect and all such aspects described herein, the concentration in the vial of each of the synthetic, modified RNAs encoding reprogramming factors is 100 ng/μl.

[00147] In some embodiments of this aspect and all such aspects described herein, the kit further comprises a vial comprising a synthetic, modified RNA molecule encoding a LIN28 reprogramming factor and a buffer.

[00148] In some embodiments of this aspect and all such aspects described herein, the buffer is RNase-free TE buffer at pH 7.0.

[00149] In some embodiments of this aspect and all such aspects described herein, the kit further a synthetic, modified RNA encoding a positive control.

[00150] In one embodiment of those aspects where a kit is provided to induce reprogramming of a somatic cell to an induced pluripotent stem cell, the kit comprises: a vial comprising a synthetic, modified RNA encoding OCT4 and a buffer; a vial comprising a synthetic, modified RNA encoding SOX2 and a buffer; a vial comprising a synthetic, modified RNA encoding c-MYC and a buffer; a vial comprising a synthetic, modified RNA encoding KLF4 and a buffer; a vial comprising a synthetic, modified RNA molecule encoding LIN28 and a buffer; a vial comprising a synthetic, modified RNA encoding a positive control GFP molecule; and packaging and instructions therefor; where the buffers in each of the vials is RNase-free TE buffer at pH 7.0; and where the synthetic, modified RNAs encoding OCT4, SOX2, c-MYC, KLF-4, LIN28 and GFP all comprise pseudouridine and 5-methylcytidine nucleoside modifications. In one embodiment, the concentration of the synthetic, modified RNAs encoding OCT4, SOX2, c-MYC, KLF-4, LIN28 and GFP in each of the vials is 100 ng/μl.

[00151] Also provided, in another aspect, is a kit for reprogramming a somatic cell to an induced pluripotent stem cell, the kit comprising: a) a container comprising a synthetic, modified RNA encoding an OCT4 reprogramming factor; a synthetic, modified RNA encoding a SOX2 reprogramming factor; a synthetic, modified RNA encoding a c-MYC reprogramming factor; a synthetic, modified RNA encoding a KLF4 reprogramming factor; and a buffer, where each of the synthetic, modified RNAs encoding a reprogramming factor comprises at least two modified nucleosides; and b) packaging and instructions therefor.

[00152] In some embodiments of this aspect and all such aspects described herein, the at least two modified nucleosides are pseudouridine and 5-methylcytodine.

[00153] In some embodiments of this aspect and all such aspects described herein, the concentration in the container of the synthetic, modified RNAs encoding reprogramming factors is 100 ng/μl.

[00154] In some embodiments of this aspect and all such aspects described herein, the kit further comprises a synthetic, modified RNA molecule encoding a LIN28 reprogramming actor.

[00155] In some embodiments of this aspect and all such aspects described herein, the kit further comprises a synthetic, modified RNA encoding a positive control.

[00156] In some embodiments of this aspect and all such aspects described herein, the buffer is RNase-free TE buffer at pH 7.0.

[00157] In some embodiments of this aspect and all such aspects described herein, each of the synthetic, modified RNAs encoding a reprogramming factor further comprise a ligand. In some such

embodiments of this aspect and all such aspects described herein, the ligand is a lipid or lipid-based molecule.

Definitions

[00158] For convenience, certain terms employed herein, in the specification, examples and appended claims are collected here. Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. Unless explicitly stated otherwise, or apparent from context, the terms and phrases below do not exclude the meaning that the term or phrase has acquired in the art to which it pertains. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed invention, because the scope of the invention is limited only by the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[00159] As used herein, the terms "developmental potential" or "developmental potency" refer to the total of all developmental cell fates or cell types that can be achieved by a cell upon differentiation. Thus, a cell with greater or higher developmental potential can differentiate into a greater variety of different cell types than a cell having a lower or decreased developmental potential. The developmental potential of a cell can range from the highest developmental potential of a totipotent cell, which, in addition to being able to give rise to all the cells of an organism, can give rise to extra-embryonic tissues; to a "unipotent cell," which has the capacity to differentiate into only one type of tissue or cell type, but has the property of self-renewal, as described herein; to a "terminally differentiated cell," which has the lowest developmental potential. A cell with "parental developmental potential" refers to a cell having the developmental potential of the parent cell that gave rise to it.

[00160] The term "totipotency" refers to a cell with a developmental potential to make all of the cells in the adult body as well as the extra-embryonic tissues, including the placenta. The fertilized egg (zygote) is totipotent, as are the cells (blastomeres) of the morula (up to the 16-cell stage following fertilization).

[00161] The term "pluripotent" as used herein refers to a cell with the developmental potential, under different conditions, to differentiate to cell types characteristic of all three germ cell layers, *i.e.*, endoderm (*e.g.*, gut tissue), mesoderm (including blood, muscle, and vessels), and ectoderm (such as skin and nerve). A pluripotent cell has a lower developmental potential than a totipotent cell. The ability of a cell to differentiate to all three germ layers can be determined using, for example, a nude mouse teratoma formation assay. In some embodiments, pluripotency can also evidenced by the expression of embryonic stem (ES) cell markers, although the preferred test for pluripotency of a cell or population of cells generated using the compositions and methods described herein is the demonstration that a cell has the developmental potential to differentiate into cells of each of the three germ layers. In some embodiments, a pluripotent cell is termed an "undifferentiated cell."

Accordingly, the terms "pluripotency" or a "pluripotent state" as used herein refer to the developmental potential of a cell that provides the ability for the cell to differentiate into all three embryonic germ layers (endoderm, mesoderm and ectoderm). Those of skill in the art are aware of the embryonic germ layer or lineage that gives rise to a given cell type. A cell in a pluripotent state typically has the potential to divide *in vitro* for a long period of time, *e.g.*, greater than one year or more than 30 passages.

[00162] The term "multipotent" when used in reference to a "multipotent cell" refers to a cell that has the developmental potential to differentiate into cells of one or more germ layers, but not all three. Thus, a multipotent cell can also be termed a "partially differentiated cell." Multipotent cells are well known in the art, and examples of multipotent cells include adult stem cells, such as for example, hematopoietic stem cells and neural stem cells. "Multipotent" indicates that a cell may form many types of cells in a given lineage, but not cells of other lineages. For example, a multipotent hematopoietic cell can form the many different types of blood cells (red, white, platelets, etc...), but it cannot form neurons. Accordingly, the term "multipotency" refers to a state of a cell with a degree of developmental potential that is less than totipotent and pluripotent.

[00163] The terms "stem cell" or "undifferentiated cell" as used herein, refer to a cell in an undifferentiated or partially differentiated state that has the property of self-renewal and has the developmental potential to differentiate into multiple cell types, without a specific implied meaning regarding developmental potential (*i.e.*, totipotent, pluripotent, multipotent, etc.). A stem cell is capable of proliferation and giving rise to more such stem cells while maintaining its developmental potential. In theory, self-renewal can occur by either of two major mechanisms. Stem cells can divide asymmetrically, which is known as obligatory asymmetrical differentiation, with one daughter cell retaining the developmental potential of the parent stem cell and the other daughter cell expressing some distinct other specific function, phenotype and/or developmental potential from the parent cell. The daughter cells themselves can be induced to proliferate and produce progeny that subsequently differentiate into one or more mature cell types, while also retaining one or more cells with parental developmental potential. A differentiated cell may derive from a multipotent cell, which itself is derived from a multipotent cell, and so on. While each of these multipotent cells may be considered stem cells, the range of cell types each such stem cell can give rise to, *i.e.*, their developmental potential, can vary considerably. Alternatively, some of the stem cells in a population can divide symmetrically into two stem cells, known as stochastic differentiation, thus maintaining some stem cells in the population as a whole, while other cells in the population give rise to differentiated progeny only. Accordingly, the term "stem cell" refers to any subset of cells that have the developmental potential, under particular circumstances, to differentiate to a more specialized or differentiated phenotype, and which retain the capacity, under certain circumstances, to proliferate without substantially differentiating. In some embodiments, the term stem cell refers generally to a naturally occurring parent cell whose descendants (progeny cells) specialize, often in different

directions, by differentiation, *e.g.*, by acquiring completely individual characters, as occurs in progressive diversification of embryonic cells and tissues. Some differentiated cells also have the capacity to give rise to cells of greater developmental potential. Such capacity may be natural or may be induced artificially upon treatment with various factors. Cells that begin as stem cells might proceed toward a differentiated phenotype, but then can be induced to "reverse" and re-express the stem cell phenotype, a term often referred to as "dedifferentiation" or "reprogramming" or "retrodifferentiation" by persons of ordinary skill in the art.

[00164] The term "embryonic stem cell" as used herein refers to naturally occurring pluripotent stem cells of the inner cell mass of the embryonic blastocyst (see, for *e.g.*, US Patent Nos. 5,843,780; 6,200,806; 7,029,913; 7,584,479, which are incorporated herein by reference). Such cells can similarly be obtained from the inner cell mass of blastocysts derived from somatic cell nuclear transfer (see, for example, US Patent Nos. 5,945,577, 5,994,619, 6,235,970). Embryonic stem cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. In other words, they can develop into each of the more than 200 cell types of the adult body when given sufficient and necessary stimulation for a specific cell type. They do not contribute to the extra-embryonic membranes or the placenta, *i.e.*, are not totipotent.

[00165] As used herein, the distinguishing characteristics of an embryonic stem cell define an "embryonic stem cell phenotype." Accordingly, a cell has the phenotype of an embryonic stem cell if it possesses one or more of the unique characteristics of an embryonic stem cell, such that that cell can be distinguished from other cells not having the embryonic stem cell phenotype. Exemplary distinguishing embryonic stem cell phenotype characteristics include, without limitation, expression of specific cell-surface or intracellular markers, including protein and microRNAs, gene expression profiles, methylation profiles, deacetylation profiles, proliferative capacity, differentiation capacity, karyotype, responsiveness to particular culture conditions, and the like. In some embodiments, the determination of whether a cell has an "embryonic stem cell phenotype" is made by comparing one or more characteristics of the cell to one or more characteristics of an embryonic stem cell line cultured within the same laboratory.

[00166] The term "somatic stem cell" is used herein to refer to any pluripotent or multipotent stem cell derived from non-embryonic tissue, including fetal, juvenile, and adult tissue. Natural somatic stem cells have been isolated from a wide variety of adult tissues including blood, bone marrow, brain, olfactory epithelium, skin, pancreas, skeletal muscle, and cardiac muscle. Each of these somatic stem cells can be characterized based on gene expression, factor responsiveness, and morphology in culture. Exemplary naturally occurring somatic stem cells include, but are not limited to, neural stem cells, neural crest stem cells, mesenchymal stem cells, hematopoietic stem cells, and pancreatic stem cells. In some aspects described herein, a "somatic pluripotent cell" refers to a somatic cell, or a progeny cell of the somatic cell, that has had its developmental potential altered, *i.e.*,

increased, to that of a pluripotent state by contacting with, or the introduction of, one or more reprogramming factors using the compositions and methods described herein.

[00167] The term "progenitor cell" is used herein to refer to cells that have greater developmental potential, *i.e.*, a cellular phenotype that is more primitive (*e.g.*, is at an earlier step along a developmental pathway or progression) relative to a cell which it can give rise to by differentiation. Often, progenitor cells have significant or very high proliferative potential. Progenitor cells can give rise to multiple distinct cells having lower developmental potential, *i.e.*, differentiated cell types, or to a single differentiated cell type, depending on the developmental pathway and on the environment in which the cells develop and differentiate.

[00168] As used herein, the term "somatic cell" refers to any cell other than a germ cell, a cell present in or obtained from a pre-implantation embryo, or a cell resulting from proliferation of such a cell *in vitro*. Stated another way, a somatic cell refers to any cell forming the body of an organism, as opposed to a germline cell. In mammals, germline cells (also known as "gametes") are the spermatozoa and ova which fuse during fertilization to produce a cell called a zygote, from which the entire mammalian embryo develops. Every other cell type in the mammalian body—apart from the sperm and ova, the cells from which they are made (gametocytes) and undifferentiated, pluripotent, embryonic stem cells—is a somatic cell: internal organs, skin, bones, blood, and connective tissue are all made up of somatic cells. In some embodiments the somatic cell is a "non-embryonic somatic cell," by which is meant a somatic cell that is not present in or obtained from an embryo and does not result from proliferation of such a cell *in vitro*. In some embodiments the somatic cell is an "adult somatic cell," by which is meant a cell that is present in or obtained from an organism other than an embryo or a fetus or results from proliferation of such a cell *in vitro*. Unless otherwise indicated, the compositions and methods for reprogramming a somatic cell described herein can be performed both *in vivo* and *in vitro* (where *in vivo* is practiced when a somatic cell is present within a subject, and where *in vitro* is practiced using an isolated somatic cell maintained in culture).

[00169] The term "differentiated cell" encompasses any somatic cell that is not, in its native form, pluripotent, as that term is defined herein. Thus, the term a "differentiated cell" also encompasses cells that are partially differentiated, such as multipotent cells, or cells that are stable, non-pluripotent partially reprogrammed, or partially differentiated cells, generated using any of the compositions and methods described herein. In some embodiments, a differentiated cell is a cell that is a stable intermediate cell, such as a non-pluripotent, partially reprogrammed cell. It should be noted that placing many primary cells in culture can lead to some loss of fully differentiated characteristics. Thus, simply culturing such differentiated or somatic cells does not render these cells non-differentiated cells (*e.g.* undifferentiated cells) or pluripotent cells. The transition of a differentiated cell (including stable, non-pluripotent partially reprogrammed cell intermediates) to pluripotency requires a reprogramming stimulus beyond the stimuli that lead to partial loss of differentiated character upon placement in culture. Reprogrammed and, in some embodiments,

partially reprogrammed cells, also have the characteristic of having the capacity to undergo extended passaging without loss of growth potential, relative to parental cells having lower developmental potential, which generally have capacity for only a limited number of divisions in culture. In some embodiments, the term "differentiated cell" also refers to a cell of a more specialized cell type (*i.e.*, decreased developmental potential) derived from a cell of a less specialized cell type (*i.e.*, increased developmental potential) (*e.g.*, from an undifferentiated cell or a reprogrammed cell) where the cell has undergone a cellular differentiation process.

[00170] The term "reprogramming" as used herein refers to a process that reverses the developmental potential of a cell or population of cells (*e.g.*, a somatic cell). Stated another way, reprogramming refers to a process of driving a cell to a state with higher developmental potential, *i.e.*, backwards to a less differentiated state. The cell to be reprogrammed can be either partially or terminally differentiated prior to reprogramming. In some embodiments of the aspects described herein, reprogramming encompasses a complete or partial reversion of the differentiation state, *i.e.*, an increase in the developmental potential of a cell, to that of a cell having a pluripotent state. In some embodiments, reprogramming encompasses driving a somatic cell to a pluripotent state, such that the cell has the developmental potential of an embryonic stem cell, *i.e.*, an embryonic stem cell phenotype. In some embodiments, reprogramming also encompasses a partial reversion of the differentiation state or a partial increase of the developmental potential of a cell, such as a somatic cell or a unipotent cell, to a multipotent state. Reprogramming also encompasses partial reversion of the differentiation state of a cell to a state that renders the cell more susceptible to complete reprogramming to a pluripotent state when subjected to additional manipulations, such as those described herein. Such manipulations can result in endogenous expression of particular genes by the cells, or by the progeny of the cells, the expression of which contributes to or maintains the reprogramming. In certain embodiments, reprogramming of a cell using the synthetic, modified RNAs and methods thereof described herein causes the cell to assume a multipotent state (*e.g.*, is a multipotent cell). In some embodiments, reprogramming of a cell (*e.g.* a somatic cell) using the synthetic, modified RNAs and methods thereof described herein causes the cell to assume a pluripotent-like state or an embryonic stem cell phenotype. The resulting cells are referred to herein as "reprogrammed cells," "somatic pluripotent cells," and "RNA-induced somatic pluripotent cells." The term "partially reprogrammed somatic cell" as referred to herein refers to a cell which has been reprogrammed from a cell with lower developmental potential by the methods as disclosed herein, wherein the partially reprogrammed cell has not been completely reprogrammed to a pluripotent state but rather to a non-pluripotent, stable intermediate state. Such a partially reprogrammed cell can have a developmental potential lower that a pluripotent cell, but higher than a multipotent cell, as those terms are defined herein. A partially reprogrammed cell can, for example, differentiate into one or two of the three germ layers, but cannot differentiate into all three of the germ layers.

[00171] The term "developmental potential altering factor," as used herein, refers to a factor such as a protein or RNA, the expression of which alters the developmental potential of a cell, *e.g.*, a somatic cell, to another developmental state, *e.g.*, a pluripotent state. Such an alteration in the developmental potential can be a decrease (*i.e.*, to a more differentiated developmental state) or an increase (*i.e.*, to a less differentiated developmental state). A developmental potential altering factor, can be for example, an RNA or protein product of a gene encoding a reprogramming factor, such as SOX2, an RNA or protein product of a gene encoding a cell-type specific polypeptide transcription factor, such as myoD, a microRNA, a small molecule, and the like.

[00172] The term a "reprogramming factor," as used herein, refers to a developmental potential altering factor, as that term is defined herein, such as a protein, RNA, or small molecule, the expression of which contributes to the reprogramming of a cell, *e.g.* a somatic cell, to a less differentiated or undifferentiated state, *e.g.* to a cell of a pluripotent state or partially pluripotent state. A reprogramming factor can be, for example, transcription factors that can reprogram cells to a pluripotent state, such as SOX2, OCT3/4, KLF4, NANOG, LIN-28, c-MYC, and the like, including as any gene, protein, RNA or small molecule, that can substitute for one or more of these in a method of reprogramming cells *in vitro*. In some embodiments, exogenous expression of a reprogramming factor, using the synthetic modified RNAs and methods thereof described herein, induces endogenous expression of one or more reprogramming factors, such that exogenous expression of one or more reprogramming factors is no longer required for stable maintenance of the cell in the reprogrammed or partially reprogrammed state. "Reprogramming to a pluripotent state *in vitro*" is used herein to refer to *in vitro* reprogramming methods that do not require and/or do not include nuclear or cytoplasmic transfer or cell fusion, *e.g.*, with oocytes, embryos, germ cells, or pluripotent cells. A reprogramming factor can also be termed a "de-differentiation factor," which refers to a developmental potential altering factor, as that term is defined herein, such as a protein or RNA, that induces a cell to de-differentiate to a less differentiated phenotype, that is a de-differentiation factor increases the developmental potential of a cell.

[00173] As used herein, the term "differentiation factor" refers to a developmental potential altering factor, as that term is defined herein, such as a protein, RNA, or small molecule, that induces a cell to differentiate to a desired cell-type, *i.e.*, a differentiation factor reduces the developmental potential of a cell. In some embodiments, a differentiation factor can be a cell-type specific polypeptide, however this is not required. Differentiation to a specific cell type can require simultaneous and/or successive expression of more than one differentiation factor. In some aspects described herein, the developmental potential of a cell or population of cells is first increased via reprogramming or partial reprogramming using synthetic, modified RNAs, as described herein, and then the cell or progeny cells thereof produced by such reprogramming are induced to undergo differentiation by contacting with, or introducing, one or more synthetic, modified RNAs encoding

differentiation factors, such that the cell or progeny cells thereof have decreased developmental potential.

[00174] In the context of cell ontogeny, the term "differentiate", or "differentiating" is a relative term that refers to a developmental process by which a cell has progressed further down a developmental pathway than its immediate precursor cell. Thus in some embodiments, a reprogrammed cell as the term is defined herein, can differentiate to a lineage-restricted precursor cell (such as a mesodermal stem cell), which in turn can differentiate into other types of precursor cells further down the pathway (such as a tissue specific precursor, for example, a cardiomyocyte precursor), and then to an end-stage differentiated cell, which plays a characteristic role in a certain tissue type, and may or may not retain the capacity to proliferate further.

[00175] As used herein, the term "cell-type specific polypeptide" refers to a polypeptide that is expressed in a cell having a particular phenotype (*e.g.*, a muscle cell, a pancreatic β cell) but is not generally expressed in other cell types with different phenotypes. As but one example, MyoD is expressed specifically in muscle cells but not in non-muscle cells, thus MyoD is a cell-type specific polypeptide.

[00176] As used herein, the term "without the formation of a pluripotent intermediate cell" refers to the transdifferentiation of one cell type to another cell type, preferably, in one step; thus a method that modifies the differentiated phenotype or developmental potential of a cell without the formation of a pluripotent intermediate cell does not require that the cell be first dedifferentiated (or reprogrammed) and then differentiated to another cell type. Instead, the cell type is merely "switched" from one cell type to another without going through a less differentiated phenotype. Accordingly, transdifferentiation refers to a change in the developmental potential of a cell whereby the cell is induced to become a different cell having a similar developmental potential, *e.g.*, a liver cell to a pancreatic cell, a pancreatic α cell into a pancreatic β cell, etc.

[00177] The term "expression" refers to the cellular processes involved in producing RNA and proteins and as appropriate, secreting proteins, including where applicable, but not limited to, for example, transcription, translation, folding, modification and processing. "Expression products" include RNA transcribed from a gene, and polypeptides obtained by translation of mRNA transcribed from a gene. In some embodiments, an expression product is transcribed from a sequence that does not encode a polypeptide, such as a microRNA.

[00178] As used herein, the term "transcription factor" refers to a protein that binds to specific parts of DNA using DNA binding domains and is part of the system that controls the transcription of genetic information from DNA to RNA.

[00179] As used herein, the term "small molecule" refers to a chemical agent which can include, but is not limited to, a peptide, a peptidomimetic, an amino acid, an amino acid analog, a polynucleotide, a polynucleotide analog, an aptamer, a nucleotide, a nucleotide analog, an organic or

inorganic compound (*e.g.*, including heterorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

[00180] The term "exogenous" as used herein refers to a nucleic acid (*e.g.*, a synthetic, modified RNA encoding a transcription factor), or a protein (*e.g.*, a transcription factor) that has been introduced by a process involving the hand of man into a biological system such as a cell or organism in which it is not normally found, or in which it is found in lower amounts. A factor (*e.g.* a synthetic, modified RNA encoding a transcription factor, or a protein, *e.g.*, a polypeptide) is considered exogenous if it is introduced into an immediate precursor cell or a progeny cell that inherits the substance. In contrast, the term "endogenous" refers to a factor or expression product that is native to the biological system or cell (*e.g.*, endogenous expression of a gene, such as, *e.g.*, SOX2 refers to production of a SOX2 polypeptide by the endogenous gene in a cell). In some embodiments, the introduction of one or more exogenous factors to a cell, *e.g.*, a developmental potential altering factor, using the compositions and methods comprising synthetic, modified RNAs described herein, induces endogenous expression in the cell or progeny cell(s) thereof of a factor or gene product necessary for maintenance of the cell or progeny cell(s) thereof in a new developmental potential.

[00181] The term "isolated" or "partially purified" as used herein refers, in the case of a nucleic acid or polypeptide, to a nucleic acid or polypeptide separated from at least one other component (*e.g.*, nucleic acid or polypeptide) that is present with the nucleic acid or polypeptide as found in its natural source and/or that would be present with the nucleic acid or polypeptide when expressed by a cell, or secreted in the case of secreted polypeptides. A chemically synthesized nucleic acid or polypeptide or one synthesized using *in vitro* transcription/translation is considered "isolated".

[00182] The term "isolated cell" as used herein refers to a cell that has been removed from an organism in which it was originally found, or a descendant of such a cell. Optionally the cell has been cultured *in vitro, e.g.*, in the presence of other cells. Optionally, the cell is later introduced into a second organism or re-introduced into the organism from which it (or the cell or population of cells from which it descended) was isolated.

[00183] The term "isolated population" with respect to an isolated population of cells as used herein refers to a population of cells that has been removed and separated from a mixed or heterogeneous population of cells. In some embodiments, an isolated population is a "substantially pure" population of cells as compared to the heterogeneous population from which the cells were isolated or enriched. In some embodiments, the isolated population is an isolated population of pluripotent cells which comprise a substantially pure population of pluripotent cells as compared to a heterogeneous population of somatic cells from which the pluripotent cells were derived.

[00184] The term "immediate precursor cell" is used herein to refer to a parental cell from which a daughter cell has arisen by cell division.

[00185] As used herein, the terms "synthetic, modified RNA" or "modified RNA" refer to an RNA molecule produced *in vitro*, which comprise at least one modified nucleoside as that term is defined herein below. The synthetic, modified RNA composition does not encompass mRNAs that are isolated from natural sources such as cells, tissue, organs etc., having those modifications, but rather only synthetic, modified RNAs that are synthesized using *in vitro* techniques. The term "composition," as applied to the terms "synthetic, modified RNA" or "modified RNA," encompasses a plurality of different synthetic, modified RNA molecules (*e.g.*, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 25, at least 30, at least 40, at least 50, at least 75, at least 90, at least 100 synthetic, modified RNA molecules or more). In some embodiments, a synthetic, modified RNA composition can further comprise other agents (*e.g.*, an inhibitor of interferon expression or activity, a transfection reagent, etc.). Such a plurality can include synthetic, modified RNA of different sequences (*e.g.*, coding for different polypeptides), synthetic, modified RNAs of the same sequence with differing modifications, or any combination thereof.

[00186] As used herein the term "modified nucleoside" refers to a ribonucleoside that encompasses modification(s) relative to the standard guanine (G), adenine (A), cytidine (C), and uridine (U) nucleosides. Such modifications can include, for example, modifications normally introduced post-transcriptionally to mammalian cell mRNA, and artificial chemical modifications, as known to one of skill in the art.

[00187] As used herein, the term " polypeptide " refers to a polymer of amino acids comprising at least 2 amino acids (*e.g.*, at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, at least 350, at least 400, at least 450, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 2000, at least 3000, at least 4000, at least 5000, at least 6000, at least 7000, at least 8000, at least 9000, at least 10,000 amino acids or more). The terms "protein" and "polypeptide" are used interchangeably herein. As used herein, the term "peptide" refers to a relatively short polypeptide, typically between about 2 and 60 amino acids in length.

[00188] As used herein, the term "added co-transcriptionally" refers to the addition of a feature, *e.g.*, a 5' diguanosine cap or other modified nucleoside, to a synthetic, modified RNA during transcription of the RNA molecule (*i.e.*, the modified RNA is not fully transcribed prior to the addition of the 5' cap).

[00189] The term "contacting" or "contact" as used herein in connection with contacting a cell with one or more synthetic, modified RNAs as described herein, includes subjecting a cell to a culture medium which comprises one or more synthetic, modified RNAs at least one time, or a

pluarlity of times, or to a method whereby such a synthetic, modified RNA is forced to contact a cell at least one time, or a pluarlity of times, *i.e.*, a transfection system. Where such a cell is *in vivo,* contacting the cell with a synthetic, modified RNA includes administering the synthetic, modified RNA in a composition, such as a pharmaceutical composition, to a subject via an appropriate administration route, such that the compound contacts the cell *in vivo.*

[00190] The term "transfection" as used herein refers the use of methods, such as chemical methods, to introduce exogenous nucleic acids, such as the synthetic, modified RNAs described herein, into a cell, preferably a eukaryotic cell. As used herein, the term transfection does not encompass viral-based methods of introducing exogenous nucleic acids into a cell. Methods of transfection include physical treatments (electroporation, nanoparticles, magnetofection), and chemical-based transfection methods. Chemical-based transfection methods include, but are not limited to, cyclodextrin, polymers, liposomes, and nanoparticles. In some embodiments, cationic lipids or mixtures thereof can be used to transfect the synthetic, modified RNAs described herein, into a cell, such as DOPA, Lipofectamine and UptiFectin. In some embodiments, cationic polymers such as DEAE-dextran or polyethylenimine, can be used to transfect a synthetic, modified RNAs described herein.

[00191] The term "transduction" as used herein refers to the use of viral particles or viruses to introduce exogenous nucleic acids into a cell.

[00192] As used herein, the term "transfection reagent" refers to any agent that induces uptake of a synthetic, modified RNA into a host cell. Also encompassed are agents that enhance uptake *e.g.*, by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 1-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 25-fold, at least 500-fold, at least 100-fold, at least 1000-fold, or more, compared to a synthetic, modified RNA administered in the absence of such a reagent. In one embodiment, a cationic or non-cationic lipid molecule useful for preparing a composition or for co-administration with a synthetic, modified RNA is used as a transfection reagent. In other embodiments, the synthetic, modified RNA comprises a chemical linkage to attach *e.g.*, a ligand, a peptide group, a lipophillic group, a targeting moiety etc. In other embodiments, the transfection reagent comprises a charged lipid, an emulsion, a liposome, a cationic or non-cationic lipid, an anionic lipid, or a penetration enhancer as known in the art or described herein.

[00193] As used herein, the term "repeated transfections" refers to repeated transfection of the same cell culture with a synthetic, modified RNA a plurality of times (*e.g.*, more than once or at least twice). In some embodiments, the cell culture is transfected at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, at least 10 times, at least 11 times, at least 12 times, at least 13 times, at least 14 times, at least 15 times, at least 16 times, at least 17 times at least 18 times, at least 19 times, at least 20 times, at least 25 times, at

least 30 times, at least 35 times, at least 40 times, at least 45 times, at least 50 times or more. The transfections can be repeated until a desired phenotype of the cell is achieved.

[00194] The time between each repeated transfection is referred to herein as the "frequency of transfection." In some embodiments, the frequency of transfection occurs every 6h, every 12h, every 24 h, every 36h, every 48h, every 60h, every 72h, every 96h, every 108h, every 5 days, every 7days, every 10 days, every 14 days, every 3 weeks, or more during a given time period in any developmental potential altering regimen, such as a reprogramming, transdifferentiation or differentiation regimen. The frequency can also vary, such that the interval between each dose is different (*e.g.*, first interval 36h, second interval 48h, third interval 72h etc). It should be understood depending upon the schedule and duration of repeated transfections, it will often be necessary to split or passage cells or change or replace the media during the transfection regimen to prevent overgrowth and replace nutrients. For the purposes of the methods described herein, transfections of a culture resulting from passaging an earlier transfected culture is considered "repeated transfection," "repeated contacting" or "contacting a plurality of times," unless specifically indicated otherwise.

[00195] As used herein, the term "permits repeated transfections" refers to a synthetic, modified RNA or synthetic, modified RNA composition that can be transfected into a given cell culture with reduced cytotoxicity compared to an RNA or RNA composition having the same sequence(s) which lacks modifications to the RNA. As used herein, the term "reduced cytotoxicity" refers to the death of less than 50% of the cells in a cell culture repeatedly transfected with a synthetic, modified RNA or synthetic, modified RNA composition, *e.g.*, less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 1%, less than 0.1% or fewer compared to transfection with a composition having the same sequence(s) but lacking modifications to the RNA. The amount of cell death in a culture can be determined using a standard Trypan Blue Exclusion assay, which turns dead cells blue while leaving living cells uncolored. Alternatively "reduced cytotoxicity" can be assessed by measuring apoptosis using *e.g.*, a TUNEL assay. Other useful measures for determining "reduced cytotoxicity" include *e.g.*, flow cytometric and bead based measurements of viability, cell growth, cellularity (measured *e.g.*, microscopically and quantitated by a hemocytometer), global protein production, secretion of cytokines (*e.g.*, Type 1 IFNs), and expression level of interferon response signature genes (*e.g.*, IFIT1, IFITM1, OAS1, IFNA1, IFNB1, PKR, RIG-I, TLR7, TLR8 etc).

[00196] As used herein, the term "targeting moiety" refers to an agent that homes to or preferentially associates or binds to a particular tissue, cell type, receptor, infecting agent or other area of interest. The addition of a targeting moiety to an RNA delivery composition will enhance the delivery of the composition to a desired cell type or location. The addition to, or expression of, a targeting moiety in a cell enhances the localization of that cell to a desired location within an animal or subject.

[00197] As used herein, the terms "innate immune response" or "interferon response" refers to a cellular defense response initiated by a cell in response to recognition of infection by a foreign

organism, such as a virus or bacteria or a product of such an organism, *e.g.*, an RNA lacking the modifications characteristic of RNAs produced in the subject cell. The innate immune response protects against viral and bacterial infection by inducing the death of cells that detect exogenous nucleic acids *e.g.*, by detection of single- or double- stranded RNA that are recognized by pattern recognition receptors such as RIG-I, protein kinase R (PKR), MDA5, or nucleic acid-recognizing Toll-like receptors, *e.g.*, TLR3, TLR7, TLR8, and TLR9, and activating an interferon response. As used herein, the innate immune response or interferon response operates at the single cell level causing cytokine expression, cytokine release, global inhibition of protein synthesis, global destruction of cellular RNA, upregulation of major histocompatbility molecules, and/or induction of apoptotic death, induction of gene transcription of genes involved in apoptosis, anti-growth, and innate and adaptive immune cell activation. Some of the genes induced by type I IFNs include PKR, ADAR (adenosine deaminase acting on RNA), OAS (2',5'-oligoadenylate synthetase), RNase L, and Mx proteins. PKR and ADAR lead to inhibition of translation initiation and RNA editing, respectively. OAS is a dsRNA-dependent synthetase that activates the endoribonuclease RNase L to degrade ssRNA.

[00198] Accordingly, as used herein, the phrases "innate immune response signature" or "interferon response signature" genes refer to the set of genes that are expressed or up-regulated upon an interferon response of a cell, and include, but are not limited to, IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, CDC20, TLR3, TLR7, TLR8, and TLR9.

[00199] As used herein, the term "inhibitor of interferon expression or activity" refers to an agent (*e.g.*, small molecule, antibody, antibody fragment, soluble receptor, RNA interference molecule etc.) that: (a) inhibits translation of an interferon polypeptide from an mRNA transcript, (b) inactivates an interferon polypeptide, (c) prevents interferon binding to its receptor or (d) binds/sequesters an interferon polypeptide *e.g.*, for degradation.

[00200] As used herein, the term "unsupervised clustering analysis" or "unsupervised cluster analysis" refers to methods used in multivariate analysis to divide up objects into similar groups, or, in some embodiments, groups whose members are all close to one another on various dimensions being measured in the various objects. In cluster analysis, one does not start with any *a priori* notion of group characteristics. As used herein, "hierarchical cluster analysis" or "hierarchical clustering" refer to a general approach to unsupervised cluster analysis, in which the purpose is to group together objects or records that are "close" to one another. A key component of the analysis is repeated calculation of distance measures between objects, and between clusters once objects begin to be grouped into clusters. The outcome is typically represented graphically as a dendrogram. Hierarchical cluster analysis can be performed using any of a variety of unbiased computational methods, algorithms and software programs known to one of skill in the art that identify clusters or natural data structures from large data sets, such as, for example, gene expression data sets. Such methods include,

but are not limited to, bottom-up hierarchical clustering, K-means clustering Affinity Propagation, non-Negative Matrix Factorization, spectral clustering, Self-Organizing Map (SOM) algorithms, and the like. In some embodiments of the aspects described herein, a SOM-based method for use in unsupervised hierarchical clustering analysis of cells contacted with the synthetic, modified RNAs described herein is the Automatic clustering using density-equalized SOM Ensembles (AUTOsome) method as described in A.M. Newman and J.B. Cooper (2010, Cell Stem Cell, 7:258-262) and A.M. Newman and J.B. Cooper (2010, BMC Bioinformatics 2010, 11:117), the contents of each of which are herein incorporated in their entireties by reference. After a clustering analysis of a given data set, such as a gene expression data set, appropriate class-based statistical tests like Student's t-test, ANOVA, or Gene Set Enrichment Analysis can be used to evaluate significance.

[00201] As used herein the term "comprising " or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the invention, yet open to the inclusion of unspecified elements, whether essential or not.

[00202] As used herein the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the invention.

[00203] The term "consisting of" refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

BRIEF DESCRIPTION OF THE FIGURES

[00204] **Figure 1** depicts a synthetic, modified RNA production flowchart. To construct a template for RNA transcription reactions, the ORF of a gene of interest is first PCR amplified from a cDNA. Long oligonucleotides containing UTR sequences are then joined to the top strand of ORF amplicons by a thermostable DNA ligase, mediated by annealing to splint oligos which bring the desired single-stranded DNA (ssDNA) ends together. An upstream T7 promoter is incorporated in the 5' UTR fragment. The ssDNA product is amplified using generic primers and TA cloned. A polyA tail is added with a PCR reaction using a T_{120}-heeled reverse primer, and the amplicons are used to template IVT reactions. Modified and unmodified nucleobases are used in the IVT reaction. An anti-reverse di-guanosine cap analog (ARCA) is included in the IVT reaction at four-fold higher concentration than guanosine triphosphate (GTP), as a result of which an estimated 80% of the product is capped. Spin-column purified IVT product is DNase-treated to eliminate the DNA template. Treatment with a phosphatase is used to remove immunonogenic 5' triphosphate moieties from the uncapped RNA fraction. The completed synthetic, modified RNA is then re-purified for use in transfections.

[00205] **Figures 2A-2L** demonstrate that synthetic, modified RNA overcomes cellular anti-viral responses and can be used to direct and alter cell fate and developmental potential. Keratinocytes

were transfected 24 hours earlier with 400 ng/well of synthetic, unmodified (No Mods), 5-methyl-cytosine modified (5mC), pseudouridine modified (Psi), or 5mC + Psi modified RNA encoding GFP. **Figure 2A** shows percent viability and **Figure 2H** depicts mean fluorescence intensity of the cells shown in Figures 2A-2D as measured by flow cytometry. **Figures 2B-2G** demonstrate quantitative RT-PCR data showing expression of six interferon-regulated genes in BJ fibroblasts 24 hours after transfection with unmodified (No Mods), or synthetic, modified (5mC + Psi) RNA encoding GFP (1200 ng/well), and vehicle and untransfected controls. **Figure 2I** depicts flow cytometry histograms showing GFP expression in keratinocytes transfected with 0-160 ng of modified RNA, 24 hours post transfection. Keratinocytes were co-transfected with synthetic, modified RNAs encoding GFP with a nuclear localization signal, and cytosolic mCherry proteins. **Figures 2J** and **2L** show growth kinetics and GFP expression of BJ fibroblasts transfected daily with unmodified, or synthetic, modified RNAs encoding a destabilized nuclear-localized GFP, and vehicle and untransfected controls for 10 days. **Figure 2K** shows immunostaining for the muscle-specific proteins myogenin and myosin heavy chain (MyHC) in murine C3H/10T1/2 cell cultures 3 days after 3 consecutive daily transfections with a synthetic, modified RNA encoding MYOD. Sustained GFP expression of synthetic, modified RNA transfected cells described in Figures 2J and 2L at day 10 of transfection was demonstrated by fluorescence imaging with bright field overlay and flow cytometry. Error bars indicate s.d., n=3 for all panels.

[00206] **Figures 3A-3E** demonstrate penetrant and sustained protein expression mediated by synthetic, modified RNA transfection in diverse human cell types, and effects on cell viability and global gene expression. **Figure 3A** depicts analysis of representative flow cytometry data showing penetrance of GFP expression 24-hour post-transfection of six human cell types transfected with 1000ng of synthetic, modified RNA encoding GFP. Cell types included: human epidermal keratinocytes (HEKs), adipose-derived stem cells (ADSCs), and four different human fibroblast types (BJ, Detroit 551, MRC-5 and dH1f). Error bars show s.d. for triplicate wells. **Figures 3B and 3D** show representative expression time courses for cells transfected with synthetic, modified RNAs encoding high- and low-stability GFP variants (eGFP and d2eGFP, respectively), assayed by flow cytometry. **Figure 3C** shows Annexin V staining at indicated days of BJ fibroblasts transfected daily over the course of 10 days. Microarray analysis of BJ fibroblasts transfected for 10 consecutive days with synthetic, modified RNA encoding GFP, vehicle, or untransfected controls was performed and heat-map data generated. A number of cell stress pathways examined demonstrated that prolonged transfection with synthetic, modified- RNA does not significantly impact the molecular profile of transfected cells beyond upregulation of a limited number of interferon/NFκB genes highlighted in Figure 3E. **Figure 3E** depicts all genes upregulated greater than 2-fold in synthetic, modified RNA transfected cells versus untransfected cells (right) or vehicle transfected (left) showing induction of

number of interferon/NFκB signaling genes consistent with the near but not absolute attenuation of interferon response shown in Figure 2D.

[00207] **Figures 4A-4C** demonstrate generation of RNA-induced pluripotent stem cells (RiPS) using the synthetic, modified RNAs described herein. Immunostaining for human KLF4, OCT4, and SOX2 proteins in keratinocytes 15 hours post-transfection with synthetic, modified RNA encoding KLF4, OCT4, or SOX2 was performed. **Figures 4A-4C** depict a time course analysis showing kinetics and stability of KLF4, OCT4, and SOX2 proteins after synthetic, modified RNA transfection, as assayed by flow cytometry following intracellular staining of reach protein. Brightfield images were taken during the derivation of RNA-iPS cells (RiPS) from dH1f fibroblasts showing early epitheliod morphology (day 6), small hES-like colonies (day 17), and appearance of mature iPS clones after mechanical picking and expansion (day 24). Immunohistochemistry data was obtained showing expression of a panel of pluripotency markers in expanded RiPS clones derived from dH1f fibroblasts, Detroit 551 (D551) and MRC-5 fetal fibroblasts, BJ post-natal fibroblasts, and cells derived from a skin biopsy taken from an adult cystic fibrosis patient (CF), shown also in high magnification. BG01 hES cells and BJ1 fibroblasts are included as positive and negative controls, respectively.

[00208] **Figure 5** demonstrates iPS-derivation from five human cell types. An expression time course of low-stability nuclear GFP after a single transfection into keratinocytes was assessed by flow cytometry. Bright-field and GFP images were taken at four different time points during a reprogramming experiment. RNA-encoding the low-stability GFP analyzed was spiked into the reprogramming cocktail (KMOSL) to visualize sustained protein expression from transfected synthetic, modified RNAs during iPS reprogramming. **A**ntibody stains were performed of independent RiPS clones derived from cells taken from an adult cystic fibrosis patient (CF cells), BJ postnatal fibroblasts, MRC-5 and Detroit 551 fetal fibroblasts, and human ES-derived dH1f fibroblasts. Figure 5C panels show cell-surface staining for SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81, and intracellular staining for OCT4 and NANOG. Control stains of BG01 hES cells, dH1f and BJ fibroblasts are shown. Additional control stains show the specificity of the secondary antibody used for the OCT4 and NANOG intracellular stains.

[00209] **Figure 6** demonstrates efficient RiPS derivation from BJ fibroblasts without passaging. Immunohistochemistry showing expression of pluripotency markers SSEA-4 and TRA-1-60 in a BJ fibroblast reprogramming experiment transfected for 16 days with 600ng per day of a KMOSL modified RNA cocktail containing a destabilized GFP spike-in was performed. Cultures were fixed for staining at day 18. 50,000 BJ cells were originally seeded onto feeder cells and went unpassaged throughout the course of the experiment. **Figure 6** shows quantification of TRA-1-60 colony count relative to the number of cells seeded.

[00210] **Figures 7A-7I** demonstrate a molecular characterization of RiPS cells. **Figure 7A** depicts a heatmap showing results of qRT-PCR analysis measuring the expression of pluripotency-

associated genes in RiPS cell lines, parental fibroblasts and viral-derived iPS cells relative to hES cell controls. **Figure 7B** depicts a heatmap showing results of OCT4 promoter methylation analysis of RiPS cell lines, parental fibroblasts, and hES cell controls. **Figures 7C-7H** demonstrate global gene expression profiles of BJ-, MRC5- and dH1F-derived RiPS cells shown in scatter plots against parental fibroblasts and hES cells with pluripotency-associated transcripts indicated. **Figure 7I** depicts a dendrogram showing unsupervised hierarchical clustering of the global expression profiles for RiPS cells, parental fibroblasts, hES cells, and virus-derived iPS cells. The similarity metric for comparison between different cell lines is indicated on the height of cluster dendrogram. One of skill in the art can use these methods to determine the similarity between a RiPS cell and a human embryonic stem cell, or to determine differences between a RiPS cell and a iPS cell made by another method. This figure indicates that a RiPS cell has a higher degree of similarity to an embryonic stem cell than iPS cells derived using retroviruses, *i.e.*, a RiPS cell has an "embryonic stem cell phenotype."

[00211] **Figure 8** demonstrates trilineage differentiation of RiPS cells. **Figure 8** shows yield and typology of blood-lineage colonies produced by directed differentiation of embryoid bodies in methylcellulose assays with RiPS clones derived from BJ, CF, D551 and MCR5 fibroblasts, and a human ES (H1) control. Immunostaining was performed and showied expression of the lineage markers Tuj1 (neuronal, ectodermal), and alpha-fetoprotein (epithelial, endodermal) in RiPS clones from 3 independent RiPS derivations subjected to directed differentiation. Hematoxylin and eosin staining of BJ- and dH1F-RiPS-derived teratomas demonstrated ectoderm (pigmented epithelia (BJ), neural rosettes (dH1F)), mesoderm (cartilage and muscle, both), and endoderm (gut-like endothelium, both). For blood formation and methylcellulose assays, n=3 for each clone. Teratoma formation and trilineage differentiation of synthetic, modified RNA derived iPS clones *in vivo* was also demonstrated.

[00212] **Figures 9A-9D** demonstrate high and surprising efficiency of pluripotency induction by synthetic, modified RNAs. TRA-1-60 horseradish peroxidase (HRP) staining was conducted at day 18 of a BJ-RiPS derivation with modified RNAs encoding KMOSL and **Figure 9A** shows frequency of TRA-1-60-positive colonies produced in the experiment relative to number of cells initially seeded. Error bars show s.d., n=6 for each condition. TRA-181 IIRP, TRA-160 immunofluorescence and Hoechst staining were performed and **Figure 9B** shows colony frequencies for dH1f-RiPS experiments done using 4-factor (KMOS) and 5-factor (KMOSL) synthetic, modified RNA cocktails under 5% O2 or ambient oxygen culture conditions quantified at day 18. Control wells were transfected with equal doses of synthetic, modified RNA encoding GFP. The kinetics and efficiency of retroviral and synthetic, modified RNA reprogramming were examined and compared. Timeline of colony formation is shown in **Figure 9C**, and TRA-1-60 HRP immuno-staining was performed and TRA-1-60 positive colony counts determined (**Figure 9D**) of dH1f cells reprogrammed using KMOS retroviruses (MOI=5 of each) or synthetic, modified RNA KMOS cocktails (n=3 for each condition).

[00213] **Figures 10A-10B** demonstrate efficient directed differentiation of RiPS cells to terminally differentiated myogenic fate using synthetic, modified RNA. **Figure 10A** shows a schematic of experimental design. Bright-field and immunostained images were obtained and showed large, multi-nucleated, myosin heavy chain (MyHC) and myogenin positive myotubes in cells fixed three days after cessation of MYOD synthetic, modified RNA transfection. Synthetic, modified RNA encoding GFP was administered to the controls. **Figure 10B** shows a penetrance of myogenic conversion relative to daily RNA dose. Black bars refer to an experiment in which cultures were plated at 10^4 cells/cm^2, grey bars to cultures plated at $5x10^3$ cells/cm^2. Error bars show s.d. for triplicate wells.

DETAILED DESCRIPTION

[00214] Described herein are novel compositions, methods, and kits for changing the phenotype of a cell or cells. These methods, compositions, and kits can be used either to express a desired protein in a cell or tissue, or to change the developmental potential or differentiated phenotype of a cell to that of another, desired cell type. Significantly, the methods and compositions described herein do not utilize exogenous DNA or viral vector-based methods for the expression of protein(s), and thus, do not cause permanent modification of the genome or unintended mutagenic effects.

RNAs and RNA modification

[00215] Described herein are synthetic, modified RNAs for changing the phenotype of a cell, such as expressing a polypeptide or altering the developmental potential. As used herein, the term "synthetic, modified RNA" refers to a nucleic acid molecule encoding a factor, such as a polypeptide, to be expressed in a host cell, which comprises at least one modified nucleoside and has at least the following characteristics as the term is used herein: (i) it can be generated by *in vitro* transcription and is not isolated from a cell; (ii) it is translatable in a mammalian (and preferably human) cell; and (iii) it does not provoke or provokes a significantly reduced innate immune response or interferon response in a cell to which it is introduced or contacted relative to a synthetic, non-modified RNA of the same sequence . A synthetic, modified RNA as described herein permits repeated transfections in a target cell; that is, a cell or cell population transfected with a synthetic, modified RNA molecule as described herein tolerates repeated transfection with such synthetic, modified RNA without significant induction of an innate immune response or interferon response. These three primary criteria for a synthetic, modified RNA molecule described above are described in greater detail below.

[00216] First, the synthetic, modified RNA must be able to be generated by *in vitro* transcription of a DNA template. Methods for generating templates are well known to those of skill in the art using standard molecular cloning techniques. An additional approach to the assembly of DNA templates that does not rely upon the presence of restriction endonuclease cleavage sites is also described herein (termed "splint-mediated ligation"). The transcribed, synthetic, modified RNA polymer can be modified further post-transcription, *e.g.*, by adding a cap or other functional group.

[00217] To be suitable for *in vitro* transcription, the modified nucleoside(s) must be recognized as substrates by at least one RNA polymerase enzyme. Generally, RNA polymerase enzymes can tolerate a range of nucleoside base modifications, at least in part because the naturally occurring G, A, U, and C nucleoside bases differ from each other quite significantly. Thus, the structure of a modified nucleoside base for use in generating the synthetic, modified RNAs described herein can generally vary more than the sugar-phosphate moieties of the modified nucleoside. That said, ribose and phosphate-modified nucleosides or nucleoside analogs are known in the art that permit transcription by RNA polymerases. In some embodiments of the aspects described herein, the RNA polymerase is a phage RNA polymerase. The modified nucleotides pseudouridine, m5U, s2U, m6A, and m5C are known to be compatible with transcription using phage RNA polymerases, while N1-methylguanosine, N1-methyladenosine, N7-methylguanosine, 2'-)-methyluridine, and 2'-O-methylcytidine are not. Polymerases that accept modified nucleosides are known to those of skill in the art.

[00218] It is also contemplated that modified polymerases can be used to generate synthetic, modified RNAs, as described herein. Thus, for example, a polymerase that tolerates or accepts a particular modified nucleoside as a substrate can be used to generate a synthetic, modified RNA including that modified nucleoside.

[00219] Second, the synthetic, modified RNA must be translatable by the translation machinery of a eukaryotic, preferably mammalian, and more preferably, human cell. Translation generally requires at least a ribosome binding site, a methionine start codon, and an open reading frame encoding a polypeptide. Preferably, the synthetic, modified RNA also comprises a 5′ cap, a stop codon, a Kozak sequence, and a polyA tail. In addition, mRNAs in a eukaryotic cell are regulated by degradation, thus a synthetic, modified RNA as described herein can be further modified to extend its half-life in the cell by incorporating modifications to reduce the rate of RNA degradation (*e.g.*, by increasing serum stability of a synthetic, modified RNA).

[00220] Nucleoside modifications can interfere with translation. To the extent that a given modification interferes with translation, those modifications are not encompassed by the synthetic, modified RNA as described herein. One can test a synthetic, modified RNA for its ability to undergo translation and translation efficiency using an *in vitro* translation assay (*e.g.*, a rabbit reticulocyte lysate assay, a reporter activity assay, or measurement of a radioactive label in the translated protein) and detecting the amount of the polypeptide produced using SDS-PAGE, Western blot, or immunochemistry assays etc. The translation of a synthetic, modified RNA comprising a candidate modification is compared to the translation of an RNA lacking the candidate modification, such that if the translation of the synthetic, modified RNA having the candidate modification remains the same or is increased then the candidate modification is contemplated for use with the compositions and methods described herein. It is noted that fluoro-modified nucleosides are generally not translatable and can be used herein as a negative control for an *in vitro* translation assay.

[00221] Third, the synthetic, modified RNA provokes a reduced (or absent) innate immune response or interferon response by the transfected cell or population of cells thereof. mRNA produced in eukaryotic cells, *e.g.*, mammalian or human cells, is heavily modified, the modifications permitting the cell to detect RNA not produced by that cell. The cell responds by shutting down translation or otherwise initiating an innate immune or interferon response. Thus, to the extent that an exogenously added RNA can be modified to mimic the modifications occurring in the endogenous RNAs produced by a target cell, the exogenous RNA can avoid at least part of the target cell's defense against foreign nucleic acids. Thus, in some embodiments, synthetic, modified RNAs as described herein include *in vitro* transcribed RNAs including modifications as found in eukaryotic/mammalian/human RNA *in vivo*. Other modifications that mimic such naturally occurring modifications can also be helpful in producing a synthetic, modified RNA molecule that will be tolerated by a cell. With this as a background or threshold understanding for the requirements of a synthetic, modified RNA, the various modifications contemplated or useful in the synthetic, modified RNAs described herein are discussed further herein below.

RNA Modifications

[00222] In some aspects, provided herein are synthetic, modified RNA molecules encoding polypeptides, wherein the synthetic, modified RNA molecules comprise one or more modifications, such that introducing the synthetic, modified RNA molecules to a cell results in a reduced innate immune response relative to a cell contacted with synthetic RNA molecules encoding the polypeptides not comprising said one or more modifications.

[00223] The synthetic, modified RNAs described herein include modifications to prevent rapid degradation by endo- and exo-nucleases and to avoid or reduce the cell's innate immune or interferon response to the RNA. Modifications include, but are not limited to, for example, (a) end modifications, *e.g.*, 5' end modifications (phosphorylation dephosphorylation, conjugation, inverted linkages, etc.), 3' end modifications (conjugation, DNA nucleotides, inverted linkages, etc.), (b) base modifications, *e.g.*, replacement with modified bases, stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, or conjugated bases, (c) sugar modifications (*e.g.*, at the 2' position or 4' position) or replacement of the sugar, as well as (d) internucleoside linkage modifications, including modification or replacement of the phosphodiester linkages. To the extent that such modifications interfere with translation (*i.e.*, results in a reduction of 50% or more in translation relative to the lack of the modification - *e.g.*, in a rabbit reticulocyte *in vitro* translation assay), the modification is not suitable for the methods and compositions described herein. Specific examples of synthetic, modified RNA compositions useful with the methods described herein include, but are not limited to, RNA molecules containing modified or non-natural internucleoside linkages. Synthetic, modified RNAs having modified internucleoside linkages include, among others, those that do not have a phosphorus atom in the internucleoside linkage. In other embodiments, the synthetic, modified RNA has a phosphorus atom in its internucleoside linkage(s).

[00224] Non-limiting examples of modified internucleoside linkages include phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those) having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

[00225] Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6, 239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and US Pat RE39464.

[00226] Modified internucleoside linkages that do not include a phosphorus atom therein have internucleoside linkages that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

[00227] Representative U.S. patents that teach the preparation of modified oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439.

[00228] Some embodiments of the synthetic, modified RNAs described herein include nucleic acids with phosphorothioate internucleoside linkages and oligonucleosides with heteroatom internucleoside linkage, and in particular --CH2-NH-CH2-, -CH2-N(CH3)-O-CH2- [known as a methylene (methylimino) or MMI], -CH2-O-N(CH3)-CH2-, -CH2-N(CH3)-N(CH3)-CH2- and -N(CH3)-CH2-CH2- [wherein the native phosphodiester internucleoside linkage is represented as -O-P-O-CH2-] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. In some embodiments, the nucleic acid sequences featured herein have morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

[00229] Synthetic, modified RNAs described herein can also contain one or more substituted sugar moieties. The nucleic acids featured herein can include one of the following at the 2' position: H (deoxyribose); OH (ribose); F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C1 to C10 alkyl or C2 to C10 alkenyl and alkynyl. Exemplary modifications include O[(CH2)nO] mCH3, O(CH2).nOCH3, O(CH2)nNH2, O(CH2) nCH3, O(CH2)nONH2, and O(CH2)nON[(CH2)nCH3)]2, where n and m are from 1 to about 10. In some embodiments, synthetic, modified RNAs include one of the following at the 2' position: C1 to C10 lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH3, OCN, Cl, Br, CN, CF3, OCF3, SOCH3, SO2CH3, ONO2, NO2, N3, NH2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an RNA, or a group for improving the pharmacodynamic properties of a synthetic, modified RNA, and other substituents having similar properties. In some embodiments, the modification includes a 2' methoxyethoxy (2'-O-CH2CH2OCH3, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., Helv. Chim. Acta, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e.*, a O(CH2)2ON(CH3)2 group, also known as 2'-DMAOE, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH2--O--CH2--N(CH2)2.

[00230] Other modifications include 2'-methoxy (2'-OCH3), 2'-aminopropoxy (2'-OCH2CH2CH2NH2) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the nucleic acid sequence, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked nucleotides and the 5' position of 5' terminal nucleotide. A synthetic, modified RNA can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application.

[00231] As non-limiting examples, synthetic, modified RNAs described herein can include at least one modified nucleoside including a 2'-O-methyl modified nucleoside, a nucleoside comprising a 5' phosphorothioate group, a 2'-amino-modified nucleoside, 2'-alkyl-modified nucleoside, morpholino nucleoside, a phosphoramidate or a non-natural base comprising nucleoside, or any combination thereof.

[00232] In some embodiments of this aspect and all other such aspects described herein, the at least one modified nucleoside is selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2' deoxyuridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine ($m6_2Am$), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I).

[00233] Alternatively, a synthetic, modified RNA can comprise at least two modified nucleosides, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20 or more, up to the entire length of the oligonucleotide. At a minimum, a synthetic, modified RNA molecule comprising at least one modified nucleoside comprises a single nucleoside with a modification as described herein. It is not necessary for all positions in a given synthetic, modified RNA to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single synthetic, modified RNA or even at a single nucleoside within a synthetic, modified RNA. However, it is preferred, but not absolutely necessary, that each occurrence of a given nucleoside in a molecule is modified (*e.g.*, each cytosine is a modified cytosine *e.g.*, 5mC). However, it is also contemplated that different occurrences of the same nucleoside can be modified in a different way in a given synthetic, modified RNA molecule (*e.g.*, some cytosines modified as 5mC, others modified as 2'-O-methylcytidine or other cytosine analog). The modifications need not be the same for each of a plurality of modified nucleosides in a synthetic, modified RNA. Furthermore, in some embodiments of the aspects described herein, a synthetic, modified RNA comprises at least two different modified nucleosides. In some such preferred embodiments of the aspects described herein, the at least two different modified nucleosides are 5-methylcytidine and pseudouridine. A synthetic, modified RNA can also contain a mixture of both modified and unmodified nucleosides.

[00234] As used herein, "unmodified" or "natural" nucleosides or nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). In some embodiments, a synthetic, modified RNA comprises at least one nucleoside ("base") modification or substitution. Modified nucleosides include other synthetic and natural nucleobases such as inosine, xanthine, hypoxanthine, nubularine, isoguanisine, tubercidine, 2-(halo)adenine, 2-(alkyl)adenine, 2-(propyl)adenine, 2 (amino)adenine, 2-(aminoalkyll)adenine, 2 (aminopropyl)adenine, 2 (methylthio) N6 (isopentenyl)adenine, 6 (alkyl)adenine, 6 (methyl)adenine, 7 (deaza)adenine, 8 (alkenyl)adenine, 8-(alkyl)adenine, 8 (alkynyl)adenine, 8 (amino)adenine, 8-(halo)adenine, 8-(hydroxyl)adenine, 8 (thioalkyl)adenine, 8-(thiol)adenine, N6-(isopentyl)adenine, N6 (methyl)adenine, N6, N6 (dimethyl)adenine, 2-(alkyl)guanine,2 (propyl)guanine, 6-(alkyl)guanine, 6 (methyl)guanine, 7 (alkyl)guanine, 7 (methyl)guanine, 7 (deaza)guanine, 8 (alkyl)guanine, 8-

(alkenyl)guanine, 8 (alkynyl)guanine, 8-(amino)guanine, 8 (halo)guanine, 8-(hydroxyl)guanine, 8 (thioalkyl)guanine, 8-(thiol)guanine, N (methyl)guanine, 2-(thio)cytosine, 3 (deaza) 5 (aza)cytosine, 3-(alkyl)cytosine, 3 (methyl)cytosine, 5-(alkyl)cytosine, 5-(alkynyl)cytosine, 5 (halo)cytosine, 5 (methyl)cytosine, 5 (propynyl)cytosine, 5 (propynyl)cytosine, 5 (trifluoromethyl)cytosine, 6-(azo)cytosine, N4 (acetyl)cytosine, 3 (3 amino-3 carboxypropyl)uracil, 2-(thio)uracil, 5 (methyl) 2 (thio)uracil, 5 (methylaminomethyl)-2 (thio)uracil, 4-(thio)uracil, 5 (methyl) 4 (thio)uracil, 5 (methylaminomethyl)-4 (thio)uracil, 5 (methyl) 2,4 (dithio)uracil, 5 (methylaminomethyl)-2,4 (dithio)uracil, 5 (2-aminopropyl)uracil, 5-(alkyl)uracil, 5-(alkynyl)uracil, 5-(allylamino)uracil, 5 (aminoallyl)uracil, 5 (aminoalkyl)uracil, 5 (guanidiniumalkyl)uracil, 5 (1,3-diazole-1-alkyl)uracil, 5-(cyanoalkyl)uracil, 5-(dialkylaminoalkyl)uracil, 5 (dimethylaminoalkyl)uracil, 5-(halo)uracil, 5-(methoxy)uracil, uracil-5 oxyacetic acid, 5 (methoxycarbonylmethyl)-2-(thio)uracil, 5 (methoxycarbonyl-methyl)uracil, 5 (propynyl)uracil, 5 (propynyl)uracil, 5 (trifluoromethyl)uracil, 6 (azo)uracil, dihydrouracil, N3 (methyl)uracil, 5-uracil (*i.e.*, pseudouracil), 2 (thio)pseudouracil,4 (thio)pseudouracil,2,4-(dithio)psuedouracil,5-(alkyl)pseudouracil, 5-(methyl)pseudouracil, 5-(alkyl)-2-(thio)pseudouracil, 5-(methyl)-2-(thio)pseudouracil, 5-(alkyl)-4 (thio)pseudouracil, 5-(methyl)-4 (thio)pseudouracil, 5-(alkyl)-2,4 (dithio)pseudouracil, 5-(methyl)-2,4 (dithio)pseudouracil, 1 substituted pseudouracil, 1 substituted 2(thio)-pseudouracil, 1 substituted 4 (thio)pseudouracil, 1 substituted 2,4-(dithio)pseudouracil, 1 (aminocarbonylethylenyl)-pseudouracil, 1 (aminocarbonylethylenyl)-2(thio)-pseudouracil, 1 (aminocarbonylethylenyl)-4 (thio)pseudouracil, 1 (aminocarbonylethylenyl)-2,4-(dithio)pseudouracil, 1 (aminoalkylaminocarbonylethylenyl)-pseudouracil, 1 (aminoalkylamino-carbonylethylenyl)-2(thio)-pseudouracil, 1 (aminoalkylaminocarbonylethylenyl)-4 (thio)pseudouracil, 1 (aminoalkylaminocarbonylethylenyl)-2,4-(dithio)pseudouracil, 1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-substituted 1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-substituted 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 7-substituted 1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 7-substituted 1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-(aminoalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 7-(aminoalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-(guanidiniumalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 7-(guanidiniumalkyl-hydroxy)-1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 1,3,5-(triaza)-2,6-(dioxa)-naphthalene, inosine, xanthine, hypoxanthine, nubularine, tubercidine, isoguanisine, inosinyl, 2-aza-inosinyl, 7-deaza-inosinyl, nitroimidazolyl, nitropyrazolyl, nitrobenzimidazolyl, nitroindazolyl, aminoindolyl, pyrrolopyrimidinyl, 3-(methyl)isocarbostyrilyl, 5-(methyl)isocarbostyrilyl, 3-(methyl)-7-(propynyl)isocarbostyrilyl, 7-(aza)indolyl, 6-(methyl)-7-(aza)indolyl, imidizopyridinyl, 9-(methyl)-imidizopyridinyl, pyrrolopyrizinyl, isocarbostyrilyl, 7-(propynyl)isocarbostyrilyl, propynyl-7-

(aza)indolyl, 2,4,5-(trimethyl)phenyl, 4-(methyl)indolyl, 4,6-(dimethyl)indolyl, phenyl, napthalenyl, anthracenyl, phenanthracenyl, pyrenyl, stilbenyl, tetracenyl, pentacenyl, difluorotolyl, 4-(fluoro)-6-(methyl)benzimidazole, 4-(methyl)benzimidazole, 6-(azo)thymine, 2-pyridinone, 5 nitroindole, 3 nitropyrrole, 6-(aza)pyrimidine, 2 (amino)purine, 2,6-(diamino)purine, 5 substituted pyrimidines, N2-substituted purines, N6-substituted purines, O6-substituted purines, substituted 1,2,4-triazoles, pyrrolo-pyrimidin-2-on-3-yl, 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, para-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, bis-ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, para-(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, ortho-(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, bis-ortho--(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, pyridopyrimidin-3-yl, 2-oxo-7-amino-pyridopyrimidin-3-yl, 2-oxo-pyridopyrimidine-3-yl, or any O-alkylated or N-alkylated derivatives thereof. Modified nucleosides also include natural bases that comprise conjugated moieties, *e.g.* a ligand. As discussed herein above, the RNA containing the modified nucleosides must be translatable in a host cell (*i.e.*, does not prevent translation of the polypeptide encoded by the modified RNA). For example, transcripts containing s2U and m6A are translated poorly in rabbit reticulocyte lysates, while pseudouridine, m5U, and m5C are compatible with efficient translation. In addition, it is known in the art that 2'-fluoro-modified bases useful for increasing nuclease resistance of a transcript, leads to very inefficient translation. Translation can be assayed by one of ordinary skill in the art using *e.g.*, a rabbit reticulocyte lysate translation assay.

[00235] Further modified nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in Int. Appl. No. PCT/US09/038425, filed March 26, 2009; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, and those disclosed by Englisch *et al.*, Angewandte Chemie, International Edition, 1991, 30, 613.

[00236] Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,457,191; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088 and U.S. Pat. No. 5,750,692.

[00237] Another modification for use with the synthetic, modified RNAs described herein involves chemically linking to the RNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the RNA. Ligands can be particularly useful where, for example, a synthetic, modified RNA is administered *in vivo*. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, Proc. Natl. Acid. Sci. USA,

1989, 86: 6553-6556), cholic acid (Manoharan *et al.*, Biorg. Med. Chem. Let., 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, Ann. N.Y. Acad. Sci., 1992, 660:306-309; Manoharan *et al.*, Biorg. Med. Chem. Let., 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, Nucl. Acids Res., 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, EMBO J, 1991, 10:1111-1118; Kabanov *et al.*, FEBS Lett., 1990, 259:327-330; Svinarchuk *et al.*, Biochimie, 1993, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al.*, Tetrahedron Lett., 1995, 36:3651-3654; Shea *et al.*, Nucl. Acids Res., 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, Nucleosides & Nucleotides, 1995, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, Tetrahedron Lett., 1995, 36:3651-3654), a palmityl moiety (Mishra *et al.*, Biochim. Biophys. Acta, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, J. Pharmacol. Exp. Ther., 1996, 277:923-937).

|00238| The synthetic, modified RNAs described herein can further comprise a 5' cap. In some embodiments of the aspects described herein, the synthetic, modified RNAs comprise a 5' cap comprising a modified guanine nucleotide that is linked to the 5' end of an RNA molecule using a 5'-5'triphosphate linkage. As used herein, the term "5' cap" is also intended to encompass other 5' cap analogs including, *e.g.*, 5' diguanosine cap, tetraphosphate cap analogs having a methylene-bis(phosphonate) moiety (see *e.g.*, Rydzik, AM et al., (2009) *Org Biomol Chem* 7(22):4763-76), dinucleotide cap analogs having a phosphorothioate modification (see *e.g.*, Kowalska, J. et al., (2008) *RNA* 14(6):1119-1131), cap analogs having a sulfur substitution for a non-bridging oxygen (see *e.g.*, Grudzien-Nogalska, E. et al., (2007) *RNA* 13(10): 1745-1755), N7-benzylated dinucleoside tetraphosphate analogs (see *e.g.*, Grudzien, E. et al., (2004) *RNA* 10(9):1479-1487), or anti-reverse cap analogs (see *e.g.*, Jemielity, J. et al., (2003) *RNA* 9(9): 1108-1122 and Stepinski, J. et al., (2001) *RNA* 7(10):1486-1495). In one such embodiment, the 5' cap analog is a 5' diguanosine cap. In some embodiments, the synthetic, modified RNA does not comprise a 5' triphosphate.

|00239| The 5' cap is important for recognition and attachment of an mRNA to a ribosome to initiate translation. The 5' cap also protects the synthetic, modified RNA from 5' exonuclease mediated degradation. It is not an absolute requirement that a synthetic, modified RNA comprise a 5' cap, and thus in other embodiments the synthetic, modified RNAs lack a 5' cap. However, due to the

longer half-life of synthetic, modified RNAs comprising a 5' cap and the increased efficiency of translation, synthetic, modified RNAs comprising a 5' cap are preferred herein.

[00240] The synthetic, modified RNAs described herein can further comprise a 5' and/or 3' untranslated region (UTR). Untranslated regions are regions of the RNA before the start codon (5') and after the stop codon (3'), and are therefore not translated by the translation machinery. Modification of an RNA molecule with one or more untranslated regions can improve the stability of an mRNA, since the untranslated regions can interfere with ribonucleases and other proteins involved in RNA degradation. In addition, modification of an RNA with a 5' and/or 3' untranslated region can enhance translational efficiency by binding proteins that alter ribosome binding to an mRNA. Modification of an RNA with a 3' UTR can be used to maintain a cytoplasmic localization of the RNA, permitting translation to occur in the cytoplasm of the cell. In one embodiment, the synthetic, modified RNAs described herein do not comprise a 5' or 3' UTR. In another embodiment, the synthetic, modified RNAs comprise either a 5' or 3' UTR. In another embodiment, the synthetic, modified RNAs described herein comprise both a 5' and a 3' UTR. In one embodiment, the 5' and/or 3' UTR is selected from an mRNA known to have high stability in the cell (*e.g.*, a murine alpha-globin 3' UTR). In some embodiments, the 5' UTR, the 3' UTR, or both comprise one or more modified nucleosides.

[00241] In some embodiments, the synthetic, modified RNAs described herein further comprise a Kozak sequence. The "Kozak sequence" refers to a sequence on eukaryotic mRNA having the consensus (gcc)gccRccAUGG (SEQ ID NO: 1481), where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'. The Kozak consensus sequence is recognized by the ribosome to initiate translation of a polypeptide. Typically, initiation occurs at the first AUG codon encountered by the translation machinery that is proximal to the 5' end of the transcript. However, in some cases, this AUG codon can be bypassed in a process called leaky scanning. The presence of a Kozak sequence near the AUG codon will strengthen that codon as the initiating site of translation, such that translation of the correct polypeptide occurs. Furthermore, addition of a Kozak sequence to a synthetic, modified RNA will promote more efficient translation, even if there is no ambiguity regarding the start codon. Thus, in some embodiments, the synthetic, modified RNAs described herein further comprise a Kozak consensus sequence at the desired site for initiation of translation to produce the correct length polypeptide. In some such embodiments, the Kozak sequence comprises one or more modified nucleosides.

[00242] In some embodiments, the synthetic, modified RNAs described herein further comprise a "poly (A) tail", which refers to a 3' homopolymeric tail of adenine nucleotides, which can vary in length (*e.g.*, at least 5 adenine nucleotides) and can be up to several hundred adenine nucleotides). The inclusion of a 3' poly(A) tail can protect the synthetic, modified RNA from degradation in the cell, and also facilitates extra-nuclear localization to enhance translation efficiency. In some embodiments, the poly(A) tail comprises between 1 and 500 adenine nucleotides; in other

embodiments the poly(A) tail comprises at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, at least 225, at least 250, at least 275, at least 300, at least 325, at least 350, at least 375, at least 400, at least 425, at least 450, at least 475, at least 500 adenine nucleotides or more. In one embodiment, the poly(A) tail comprises between 1 and 150 adenine nucleotides. In another embodiment, the poly(A) tail comprises between 90 and 120 adenine nucleotides. In some such embodiments, the poly(A) tail comprises one or more modified nucleosides.

[00243] It is contemplated that one or more modifications to the synthetic, modified RNAs described herein permit greater stability of the synthetic, modified RNA in a cell. To the extent that such modifications permit translation and either reduce or do not exacerbate a cell's innate immune or interferon response to the synthetic, modified RNA with the modification, such modifications are specifically contemplated for use herein. Generally, the greater the stability of a synthetic, modified RNA, the more protein can be produced from that synthetic, modified RNA. Typically, the presence of AU-rich regions in mammalian mRNAs tend to destabilize transcripts, as cellular proteins are recruited to AU-rich regions to stimulate removal of the poly(A) tail of the transcript. Loss of a poly(A) tail of a synthetic, modified RNA can result in increased RNA degradation. Thus, in one embodiment, a synthetic, modified RNA as described herein does not comprise an AU-rich region. In particular, it is preferred that the 3' UTR substantially lacks AUUUA sequence elements.

[00244] In one embodiment, a ligand alters the cellular uptake, intracellular targeting or half-life of a synthetic, modified RNA into which it is incorporated. In some embodiments a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, intracellular compartment, *e.g.*, mitochondria, cytoplasm, peroxisome, lysosome, as, *e.g.*, compared to a composition absent such a ligand. Preferred ligands do not interfere with expression of a polypeptide from the synthetic, modified RNA.

[00245] Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polylysine (PLL), poly L aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

[00246] Ligands can also include targeting groups, *e.g.*, a cell targeting agent, (*e.g.*, a lectin, glycoprotein, lipid or protein), or an antibody, that binds to a specified cell type such as a fibroblast cell. A targeting group can be, for example, a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, biotin, or an RGD peptide or RGD peptide mimetic, among others.

[00247] Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine)and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, [MPEG]2, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), and transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid).

[00248] Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a fibroblast cell, or other cell useful in the production of polypeptides. Ligands can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, or multivalent fucose.

[00249] The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the synthetic, modified RNA or a composition thereof into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, and/or intermediate filaments. The drug can be, for example, taxol, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

[00250] One exemplary ligand is a lipid or lipid-based molecule. A lipid or lipid-based ligand can (a) increase resistance to degradation, and/or (b) increase targeting or transport into a target cell or cell membrane. A lipid based ligand can be used to modulate, *e.g.*, binding of the modified RNA composition to a target cell.

[00251] In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a host cell. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include B vitamin, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up, for example, by cancer cells. Also included are HSA and low density lipoprotein (LDL).

[00252] In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-permeation agent. Preferably, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent is preferably an alpha-helical agent, which preferably has a lipophilic and a lipophobic phase.

[00253] A "cell permeation peptide" is capable of permeating a cell, *e.g.*, a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α-helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β-defensin or bactenecin), or a peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni et al., Nucl. Acids Res. 31:2717-2724, 2003).

Synthesis of synthetic, modified RNAs

[00254] The synthetic, modified RNAs described herein can be synthesized and/or modified by methods well established in the art, such as those described in "Current Protocols in Nucleic Acid Chemistry," Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA. Transcription methods are described further herein in the Examples.

[00255] In one embodiment of the aspects described herein, a template for a synthetic, modified RNA is synthesized using "splint-mediated ligation," which allows for the rapid synthesis of DNA constructs by controlled concatenation of long oligos and/or dsDNA PCR products and without the need to introduce restriction sites at the joining regions. It can be used to add generic untranslated regions (UTRs) to the coding sequences of genes during T7 template generation. Splint mediated ligation can also be used to add nuclear localization sequences to an open reading frame, and to make dominant-negative constructs with point mutations starting from a wild-type open reading frame. Briefly, single-stranded and/or denatured dsDNA components are annealed to splint oligos which bring the desired ends into conjunction, the ends are ligated by a thermostable DNA ligase and the desired constructs amplified by PCR. A synthetic, modified RNA is then synthesized from the template using an RNA polymerase *in vitro*. After synthesis of a synthetic, modified RNA is complete, the DNA template is removed from the transcription reaction prior to use with the methods described herein.

[00256] In some embodiments of these aspects, the synthetic, modified RNAs are further treated with an alkaline phosphatase.

Plurality of synthetic, modified RNAs

[00257] In some embodiments of the aspects described herein, a plurality of different synthetic, modified RNAs are contacted with, or introduced to, a cell, population of cells, or cell

culture and permit expression of at least two polypeptide products in the cell. In some embodiments, synthetic, modified RNA compositions comprise two or more synthetic, modified RNAs, *e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more synthetic, modified RNAs. In some embodiments, the two or more synthetic, modified RNAs are capable of increasing expression of a desired polypeptide product (*e.g.*, a transcription factor, a cell surface marker, a death receptor, etc.).

[00258] In some embodiments, when a plurality of different synthetic, modified RNAs, synthetic, modified RNA compositions, or media comprising a plurality of different synthetic, modified RNAs are used to modulate expression of a desired set of polypeptides, the plurality of synthetic, modified RNAs can be contacted with, or introduced to, a cell, population of cells, or cell culture simultaneously. In other embodiments, the plurality of synthetic, modified RNAs can be contacted with, or introduced to, a cell, population of cells, or cell culture separately. In addition, each synthetic, modified RNA can be administered according to its own dosage regime. For example, in one embodiment, a composition can be prepared comprising a plurality of synthetic, modified RNAs, in differing relative amounts or in equal amounts, that is contacted with a cell such that the plurality of synthetic, modified RNAs are administered simultaneously. Alternatively, one synthetic, modified RNA at a time can be administered to a cell culture (*e.g.*, sequentially). In this manner, the expression desired for each target polypeptide can be easily tailored by altering the frequency of administration and/or the amount of a particular synthetic, modified RNA administered. Contacting a cell with each synthetic, modified RNA separately can also prevent interactions between the synthetic, modified RNAs that can reduce efficiency of expression. For ease of use and to prevent potential contamination, it is preferred to administer to or contact a cell, population of cells, or cell culture with a cocktail of different synthetic, modified RNAs, thereby reducing the number of doses required and minimizing the chance of introducing a contaminant to the cell, population of cells, or cell culture.

[00259] The methods and compositions described herein permit the expression of one or more polypeptides to be tuned to a desired level by varying the amount of each synthetic, modified RNA transfected. One of skill in the art can easily monitor the expression level of the polypeptide encoded by a synthetic, modified RNA using *e.g.*, Western blotting techniques or immunocytochemistry techniques. A synthetic, modified RNA can be administered at a frequency and dose that permit a desired level of expression of the polypeptide. Each different synthetic, modified RNA can be administered at its own dose and frequency to permit appropriate expression. In addition, since the synthetic, modified RNAs administered to the cell are transient in nature (*i.e.*, are degraded over time) one of skill in the art can easily remove or stop expression of a synthetic, modified RNA by halting further transfections and permitting the cell to degrade the synthetic, modified RNA over time. The synthetic, modified RNAs will degrade in a manner similar to cellular mRNAs.

Introducing a synthetic, modified RNA into a cell

[00260] A synthetic, modified RNA can be introduced into a cell in any manner that achieves intracellular delivery of the synthetic, modified RNA, such that expression of the polypeptide encoded

by the synthetic, modified RNA can occur. As used herein, the term "transfecting a cell" refers to the process of introducing nucleic acids into cells using means for facilitating or effecting uptake or absorption into the cell, as is understood by those skilled in the art. As the term is used herein, "transfection" does not encompass viral- or viral particle based delivery methods. Absorption or uptake of a synthetic, modified RNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. Further approaches are described herein below or known in the art.

[00261] A synthetic, modified RNA can be introduced into a target cell, for example, by transfection, nucleofection, lipofection, electroporation (see, *e.g.*, Wong and Neumann, Biochem. Biophys. Res. Commun. 107:584-87 (1982)), microinjection (*e.g.*, by direct injection of a synthetic, modified RNA), biolistics, cell fusion, and the like. In an alternative embodiment, a synthetic, modified RNA can be delivered using a drug delivery system such as a nanoparticle, a dendrimer, a polymer, a liposome, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of a synthetic, modified RNA (negatively charged polynucleotides) and also enhances interactions at the negatively charged cell membrane to permit efficient cellular uptake. Cationic lipids, dendrimers, or polymers can either be bound to modified RNAs, or induced to form a vesicle or micelle (see *e.g.*, Kim SH., et al (2008) Journal of Controlled Release 129(2):107-116) that encases the modified RNA. Methods for making and using cationic-modified RNA complexes are well within the abilities of those skilled in the art (see *e.g.*, Sorensen, DR., et al (2003) J. Mol. Biol 327:761-766; Verma, UN., et al (2003) Clin. Cancer Res. 9:1291-1300; Arnold, AS et al (2007) J. Hypertens. 25:197-205).

[00262] In some embodiments of the aspects described herein, the composition further comprises a reagent that facilitates uptake of a synthetic, modified RNA into a cell (transfection reagent), such as an emulsion, a liposome, a cationic lipid, a non-cationic lipid, an anionic lipid, a charged lipid, a penetration enhancer or alternatively, a modification to the synthetic, modified RNA to attach *e.g.*, a ligand, peptide, lipophillic group, or targeting moiety.

[00263] The process for delivery of a synthetic, modified RNA to a cell will necessarily depend upon the specific approach for transfection chosen. One preferred approach is to add the RNA, complexed with a cationic transfection reagent (see below) directly to the cell culture media for the cells.

[00264] It is also contemplated herein that a first and second synthetic, modified RNA are administered in a separate and temporally distinct manner. Thus, each of a plurality of synthetic, modified RNAs can be administered at a separate time or at a different frequency interval to achieve the desired expression of a polypeptide. Typically, 100 fg to 100 pg of a synthetic, modified RNA is administered per cell using cationic lipid-mediated transfection. Since cationic lipid-mediated transfection is highly inefficient at delivering synthetic, modified RNAs to the cytosol, other techniques can require less RNA. The entire transcriptome of a mammalian cell constitutes about 1

pg of mRNA, and a polypeptide (*e.g.*, a transcription factor) can have a physiological effect at an abundance of less than 1 fg per cell.

Transfection Reagents

[00265] In certain embodiments of the aspects described herein, a synthetic, modified RNA can be introduced into target cells by transfection or lipofection. Suitable agents for transfection or lipofection include, for example, calcium phosphate, DEAE dextran, lipofectin, lipofectamine, DIMRIE C™, Superfect™, and Effectin™ (Qiagen™), unifectin™, maxifectin™, DOTMA, DOGS™ (Transfectam; dioctadecylamidoglycylspermine), DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), DOTAP (1,2-dioleoyl-3-trimethylammonium propane), DDAB (dimethyl dioctadecylammonium bromide), DHDEAB (N,N-di-n-hexadecyl-N,N-dihydroxyethyl ammonium bromide), HDEAB (N-n-hexadecyl-N,N-dihydroxyethylammonium bromide), polybrene, poly(ethylenimine) (PEI), and the like. (See, *e.g.*, Banerjee et al., Med. Chem. 42:4292-99 (1999); Godbey et al., Gene Ther. 6:1380-88 (1999); Kichler et al., Gene Ther. 5:855-60 (1998); Birchaa et al., J. Pharm. 183:195-207 (1999)).

[00266] A synthetic, modified RNA can be transfected into target cells as a complex with cationic lipid carriers (*e.g.*, Oligofectamine™) or non-cationic lipid-based carriers (*e.g.*, Transit-TKOTM™, Mirus Bio LLC, Madison, WI). Successful introduction of a modified RNA into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Successful transfection of a modified RNA can also be determined by measuring the protein expression level of the target polypeptide by *e.g.*, Western Blotting or immunocytochemistry.

[00267] In some embodiments of the aspects described herein, the synthetic, modified RNA is introduced into a cell using a transfection reagent. Some exemplary transfection reagents include, for example, cationic lipids, such as lipofectin (Junichi *et al*, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo *et al.*, PCT Application WO 97/30731). Examples of commercially available transfection reagents include, for example Lipofectamine™ (Invitrogen; Carlsbad, CA), Lipofectamine 2000™ (Invitrogen; Carlsbad, CA), 293fectin™ (Invitrogen; Carlsbad, CA), Cellfectin™ (Invitrogen; Carlsbad, CA), DMRIE-C™ (Invitrogen; Carlsbad, CA), FreeStyle™ MAX (Invitrogen; Carlsbad, CA), Lipofectamine™ 2000 CD (Invitrogen; Carlsbad, CA), Lipofectamine™ (Invitrogen; Carlsbad, CA), RNAiMAX (Invitrogen; Carlsbad, CA), Oligofectamine™ (Invitrogen; Carlsbad, CA), Optifect™ (Invitrogen; Carlsbad, CA), X-tremeGENE Q2 Transfection Reagent (Roche; Grenzacherstrasse, Switzerland), DOTAP Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), DOSPER Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), or Fugene (Grenzacherstrasse, Switzerland), Transfectam® Reagent (Promega; Madison, WI), TransFast™ Transfection Reagent (Promega; Madison, WI), Tfx™-20 Reagent (Promega; Madison, WI), Tfx™-50 Reagent (Promega; Madison, WI),

DreamFect™ (OZ Biosciences; Marseille, France), EcoTransfect (OZ Biosciences; Marseille, France), TransPass[a] D1 Transfection Reagent (New England Biolabs; Ipswich, MA, USA), LyoVec™/LipoGen™ (Invitrogen; San Diego, CA, USA), PerFectin Transfection Reagent (Genlantis; San Diego, CA, USA), NeuroPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), GenePORTER Transfection reagent (Genlantis; San Diego, CA, USA), GenePORTER 2 Transfection reagent (Genlantis; San Diego, CA, USA), Cytofectin Transfection Reagent (Genlantis; San Diego, CA, USA), BaculoPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), TroganPORTER™ transfection Reagent (Genlantis; San Diego, CA, USA), RiboFect (Bioline; Taunton, MA, USA), PlasFect (Bioline; Taunton, MA, USA), UniFECTOR (B-Bridge International; Mountain View, CA, USA), SureFECTOR (B-Bridge International; Mountain View, CA, USA), or HiFect™ (B-Bridge International, Mountain View, CA, USA), among others.

[00268] In other embodiments, highly branched organic compounds, termed "dendrimers," can be used to bind the exogenous nucleic acid, such as the synthetic, modified RNAs described herein, and introduce it into the cell.

[00269] In other embodiments of the aspects described herein,, non-chemical methods of transfection are contemplated. Such methods include, but are not limited to, electroporation (methods whereby an instrument is used to create micro-sized holes transiently in the plasma membrane of cells under an electric discharge), sono-poration (transfection via the application of sonic forces to cells), and optical transfection (methods whereby a tiny (~1 μm diameter) hole is transiently generated in the plasma membrane of a cell using a highly focused laser). In other embodiments, particle-based methods of transfections are contemplated, such as the use of a gene gun, whereby the nucleic acid is coupled to a nanoparticle of an inert solid (commonly gold) which is then "shot" directly into the target cell's nucleus; "magnetofection," which refers to a transfection method, that uses magnetic force to deliver exogenous nucleic acids coupled to magnetic nanoparticles into target cells; "impalefection," which is carried out by impaling cells by elongated nanostructures, such as carbon nanofibers or silicon nanowires which have been coupled to exogenous nucleic acids.

[00270] Other agents may be utilized to enhance the penetration of the administered nucleic acids, including glycols, such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes, such as limonene and menthone.

Synthetic, modified RNA compositions

[00271] In some embodiments of the aspects described herein, particularly embodiments involving *in vivo* administration of synthetic, modified RNAs or compositions thereof, the synthetic, modified RNAs described herein are formulated in conjunction with one or more penetration enhancers, surfactants and/or chelators. Suitable surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid,

sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.,* sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether.

[00272] The compositions described herein can be formulated into any of many possible administration forms, including a sustained release form. In some preffered embodiments of the aspects described herein, formulations comprising a plurality of different synthetic, modified RNAs are prepared by first mixing all members of a plurality of different synthetic, modified RNAs, and then complexing the mixture comprising the plurality of different synthetic, modified RNAs with a desired ligand or targeting moiety, such as a lipid. The compositions can be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions can further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension can also contain stabilizers.

[00273] The compositions described herein can be prepared and formulated as emulsions for the delivery of synthetic, modified RNAs. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1μm in diameter (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi et al., in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain further components in addition to the dispersed phases, and the active drug (*i.e.*, synthetic, modified RNA) which can be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes,

and anti-oxidants can also be present in emulsions as needed. Emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

[00274] Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

[00275] A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

[00276] Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

[00277] As noted above, liposomes can optionally be prepared to contain surface groups to facilitate delivery of liposomes and their contents to specific cell populations. For example, a liposome can comprise a surface groups such as antibodies or antibody fragments, small effector molecules for interacting with cell-surface receptors, antigens, and other like compounds.

[00278] Surface groups can be incorporated into the liposome by including in the liposomal lipids a lipid derivatized with the targeting molecule, or a lipid having a polar-head chemical group that can be derivatized with the targeting molecule in preformed liposomes. Alternatively, a targeting moiety can be inserted into preformed liposomes by incubating the preformed liposomes with a ligand-polymer-lipid conjugate.

[00279] A number of liposomes comprising nucleic acids are known in the art. WO 96/40062 (Thierry et al.) discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Pat. No. 5,264,221 (Tagawa et al.) discloses protein-bonded liposomes and asserts that the contents of such liposomes can include an RNA molecule. U.S. Pat. No. 5,665,710 (Rahman et al.) describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 (Love et al.) discloses liposomes comprising RNAi molecules targeted to the raf gene. In addition, methods for preparing a liposome composition comprising a nucleic acid can be found in *e.g.*, U.S. Patent Nos. 6,011,020; 6,074,667; 6,110,490; 6,147,204; 6, 271, 206; 6,312,956; 6,465,188; 6,506,564; 6,750,016; and 7,112,337. Each of these approaches can provide delivery of a synthetic, modified RNA as described herein to a cell.

[00280] In some embodiments of the aspects described herein, the synthetic, modified RNA described herein can be encapsulated in a nanoparticle. Methods for nanoparticle packaging are well known in the art, and are described, for example, in Bose S, et al (Role of Nucleolin in Human Parainfluenza Virus Type 3 Infection of Human Lung Epithelial Cells. J. Virol. 78:8146. 2004); Dong Y et al. Poly(d,l-lactide-co-glycolide)/montmorillonite nanoparticles for oral delivery of anticancer drugs. Biomaterials 26:6068. 2005); Lobenberg R. et al (Improved body distribution of 14C-labelled AZT bound to nanoparticles in rats determined by radioluminography. J Drug Target 5:171.1998); Sakuma S R et al (Mucoadhesion of polystyrene nanoparticles having surface hydrophilic polymeric chains in the gastrointestinal tract. Int J Pharm 177:161. 1999); Virovic L et al. Novel delivery methods for treatment of viral hepatitis: an update. Expert Opin Drug Deliv 2:707.2005); and Zimmermann E et al, Electrolyte- and pH-stabilities of aqueous solid lipid nanoparticle (SLN) dispersions in artificial gastrointestinal media. Eur J Pharm Biopharm 52:203. 2001), the contents of which are herein incoporated in their entireties by reference.

Methods for further avoiding a cell's innate immune or interferon response

[00281] Importantly, the inventors have discovered that the synthetic, modified RNAs described herein are significantly less cytotoxic when transfected into cells than their synthetic, unmodified RNA counterparts having the same nucleic acid sequence (as measured using *e.g.*, TUNEL assay or simply monitoring cellularity after transfection), which permits repeated transfections of the cells for the duration necessary to express a polypeptide in a cell, or alter the phenotype or developmental fate of the cell. The decrease in cytotoxicity stems, in part, from the presence of modified nucleoside(s) in the RNA, which reduce or prevent the development of a cellular interferon response. In some embodiments of the aspects described herein, the cellular innate immune

or interferon response comprises expression of a Type I or Type II interferon. In some embodiments of the aspects described herein, the cellular innate immune response comprises expression of one or more IFN signature genes selected from the group consisting of IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20. As noted herein, such modifications for reducing or preventing the cellular innate response include, but are not limited to, 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2' deoxyuridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine ($m6_2Am$), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In some preferred embodiments, the modifications comprise 5-methylcytidine and pseudouridine.

[00282] However, the cells transfected with the synthetic, modified RNA compositions described herein can further be treated or used with other measures to prevent or reduce any remaining cytotoxicity caused by the transfection procedure, the synthetic, modified RNAs, or a combination thereof. The cytotoxicity of synthetic, unmodified RNAs involves a cellular innate immune response designed to recognize a foreign pathogen (*e.g.*, virus) and to produce interferons, which in turn stimulates the activity of the protein kinase PKR, Toll-like receptors (TLRs) and RIG-1, among others, to mediate anti-viral actions. A significant part of an individual cell's innate immune response to foreign RNA is represented by the so-called "PKR response" triggered largely by double-stranded RNA. To the extent that all or part of the PKR response pathway can be activated by foreign single-stranded RNA, such as synthetic, modified RNAs described herein, the response is discussed herein below.

[00283] Double stranded RNA dependent protein kinase (PKR) is a member of a family of kinases that phosphorylates the alpha subunit of protein synthesis initiation factor, eIF-2 (eIF-2α) and plays a role in the translational down regulation of gene expression (Clemens et al. Mol. Biol. Rep. 1994; vol. 19: 210-10). Activation of PKR involves two molecules binding in tandem to double stranded RNA and then phosphorylating each other in an intramolecular event. (Wu et al. 1997, J. Biol. Chem 272:1291-1296). PKR has been implicated in processes that rely on apoptosis as control mechanisms *in vivo* including antiviral activities, cell growth regulation and tumorigenesis (Donze et al. EMBO J. 1995, vol. 14: 3828-34; Lee et al. Virology 1994, vol. 199: 491-6; Jagus et al. Int. J. Biochem. Cell. Biol. 1989, vol. 9: 1576-86). Regulation of protein synthesis through activated PKR arises from the interaction of PKR with foreign RNA.

[00284] It has been shown that the PKR response can be reduced by removing the 5'-triphosphate on an RNA molecule, and that RNAs having a 5'-monophosphate, -diphosphate or -7-methyl guanosine cap do not activate PKR. Thus, in one embodiment, the synthetic, modified RNA described herein comprises a 5'-monophosphate, a 5'-diphosphate, or a 5' 7-methyl guanosine cap to

escape the immune response initiated by PKR. In another embodiment, the synthetic, modified RNA as described herein is treated to remove the 5'-triphosphate using an alkaline phosphatase, *e.g.*, calf intestinal phosphatase. Other modifications to prevent activation of the immune response mediators (*e.g.*, PKR, TLRs, and RIG-1) are discussed in detail in Nallagatla, SR, et al., (2008) *RNA Biol* 5(3):140-144.

[00285] TLR7 is known to recognize single stranded RNA and binds exogenous RNAs, such as viral single-stranded RNAs in endosomes. Modifications to the RNA that reduce recognition and/or signaling by TLR7 can reduce this aspect of the innate immune response to the RNA. TLR7 signals through MyD88 and can activate a type I IFN pathway as well as an NF-κB/IL-8 pathway.

[00286] In one embodiment, the innate immune response or interferon response can be further decreased in cells transfected with a synthetic, modified RNA as described herein by co-transfection of a dominant negative mutant of a protein involved in the immunity pathways, such as RIG-1, MYD88, VISA, PKR and Toll-like receptors. Alternatively, RNA interference (*e.g.*, siRNA, shRNA, etc.) can be used to inhibit expression of RIG-1, MYD88, VISA, PKR, TRIF, TRL7, or TLR8, which will result in a lower innate immune mediated response in the cells.

[00287] Another approach to reduce the innate immune mediated response is to inhibit the effect of secreted interferon on cellular receptors, for example, by scavenging secreted interferon using a soluble interferon receptor (*e.g.*, B18R) or a neutralizing antibody. In one embodiment, a modified RNA encoding an interferon scavenging agent (*e.g.*, a soluble interferon receptor) can be administered to cells to further reduce the innate immune response of the cells.

[00288] In one embodiment, the cells transfected with synthetic, modified RNA as described herein can be grown with genetically-engineered feeder cells that secrete B18R or neutralizing antibodies to type-1 interferons.

[00289] Small molecules that inhibit the innate immune response in cells, such as chloroquine (a TLR signaling inhibitor) and 2-aminopurine (a PKR inhibitor), can also be administered into the culture media of cells transfected with the synthetic, modified RNAs described herein. Some non-limiting examples of commercially available TLR-signaling inhibitors include BX795, chloroquine, CLI-095, OxPAPC, polymyxin B, and rapamycin (all available for purchase from INVIVOGEN™). In addition, inhibitors of pattern recognition receptors (PRR) (which are involved in innate immunity signaling) such as 2-aminopurine, BX795, chloroquine, and H-89, can also be used in the compositions and methods described herein. Media supplementation with cell-penetrating peptides that inhibit proteins in the immunity pathways described above can also be combined with the use of synthetic, modified RNAs provided herein. Some non-limiting examples of commercially available cell-penetrating peptides include Pepin-MYD (INVIVOGEN™) or Pepinh-TRIF (INVIVOGEN™). An oligodeoxynucleotide antagonist for the Toll-like receptor signaling pathway can also be added to the cell culture media to reduce immunity signaling.

[00290] Another method for reducing the immune response of a cell transfected with the synthetic, modified RNAs described herein is to co-transfect mRNAs that encode negative regulators of innate immunity such as NLRX1. Alternatively, one can co-transfect viral proteins known to modulate host cell defenses such as NS1, NS3/4A, or A46R.

[00291] In another embodiment, a synthetic, modified RNA composition encoding inhibitors of the innate immune system can be used to avoid the innate immune response generated in the cell.

[00292] It is also contemplated herein that, in some embodiments, in a research setting one of skill in the art can avoid the innate immune response generated in the cell by using cells genetically deficient in antiviral pathways (*e.g.*, VISA knockout cells).

[00293] Since induction of the innate immune response results in cytokine release and death of the cells in culture, one can determine the extent of activation of an innate immune or interferon response by measuring *e.g.*, apoptosis (using *e.g.*, a TUNEL assay), reduced growth rate, reduced cellularity, reduction in global protein production, or secretion of cytokines (*e.g.*, type-I interferons such as IFN-alpha and IFN-beta, type II interferons, such as IFNγ), or upregulation of interferon stimulated genes or interferon signature genes (*e.g.*, IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20. The level of cytokine release or cell death in a transfected cell culture treated with one of the above measures described for further reducing the innate immune response can be compared to the level of an equivalent cell culture not treated to further reduce the innate immune response.

Cell Types

[00294] Provided herein are cells contacted with a synthetic, modified RNA molecule encoding a polypeptide, or a progeny cell of the contacted cell, where the synthetic, modified RNA molecule comprises one or more modifications, such that introducing the synthetic, modified RNA molecule to the cell results in a reduced innate immune response relative to the cell contacted with a synthetic RNA molecule encoding the polypeptide not comprising the one or more modifications. In some embodiments of these aspects, at least two nucleosides are modified. In some embodiments of the aspects described herein, the cellular innate immune or interferon response comprises expression of a Type I or Type II interferon. In some embodiments of the aspects described herein, the cellular innate immune response comprises expression of one or more IFN signature genes selected from the group consisting of IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20. As described herein, such modifications for reducing or preventing the cellular innate immune response include, but are not limited to, 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2' deoxyuridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A),

N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine ($m6_2Am$), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In some preferred embodiments, the modifications comprise 5-methylcytidine and pseudouridine.

[00295] Essentially any cell type can be transfected with synthetic, modified RNAs as described herein to alter the phenotype of the cell. Thus, differentiated somatic cells and stem cells, as well of cells of a cell line, can be transfected with synthetic, modified RNA as described herein. Provided herein are exemplary somatic cells, stem cells, and cell line sources useful with the methods and compositions described herein. However, the description herein is not meant to be limiting and any cell known or used in the art can be phenotypically modified by introducing one or more synthetic, modified RNAs as described herein. In embodiments relating to tissue regeneration or transplantation in a subject, the cells can be from an autologous, *i.e.*, from the same subject, or from heterologous sources.

Somatic cells

[00296] Essentially any primary somatic cell type can be used in the preparation of cells with an altered phenotype or altered developmental potential described herein. Some non-limiting examples of primary cells include, but are not limited to, fibroblast, epithelial, endothelial, neuronal, adipose, cardiac, skeletal muscle, immune cells, hepatic, splenic, lung, circulating blood cells, gastrointestinal, renal, bone marrow, and pancreatic cells. The cell can be a primary cell isolated from any somatic tissue including, but not limited to, brain, liver, lung, gut, stomach, intestine, fat, muscle, uterus, skin, spleen, endocrine organ, bone, etc. The term "somatic cell" further encompasses primary cells grown in culture, provided that the somatic cells are not immortalized.

[00297] Where the cell is maintained under *in vitro* conditions, conventional tissue culture conditions and methods can be used, and are known to those of skill in the art. Isolation and culture methods for various cells are well within the abilities of one skilled in the art.

[00298] Further, the parental cell can be from any mammalian species, with non-limiting examples including a murine, bovine, simian, porcine, equine, ovine, or human cell. In some embodiments, the cell is a human cell. In an alternate embodiment, the cell is from a non-human organism such as a non-human mammal.

Stem cells

[00299] One of the most intriguing aspects of the technologies comprising the synthetic, modified RNAs described herein is the ability to use such synthetic, modified RNAs to both generate a stem cell from a differentiated cell, and to then direct the differentiation of the stem cell to one or more desired cell types.

[00300] Stem cells are undifferentiated cells defined by their ability at the single cell level to both self-renew and differentiate to produce progeny cells, including self-renewing progenitors, non-renewing progenitors, and terminally differentiated cells. Stem cells, depending on their level of

differentiation, are also characterized by their ability to differentiate *in vitro* into functional cells of various cell lineages from multiple germ layers (endoderm, mesoderm and ectoderm), as well as to give rise to tissues of multiple germ layers following transplantation and to contribute substantially to most, if not all, tissues following injection into blastocysts. (See, *e.g.*, Potten et al., Development 110: 1001 (1990); U.S. Pat. Nos. 5,750,376, 5,851,832, 5,753,506, 5,589,376, 5,824,489, 5,654,183, 5,693,482, 5,672,499, and 5,849,553). The stem cells for use with the compositions and methods comprising synthetic, modified RNAs described herein can be naturally occurring stem cells or "induced" stem cells generated using the compositions, kits, and methods described herein, or by any method or composition known to one of skill in the art.

[00301] It is specifically noted that stem cells are useful not only for exploiting their differentiation potential to make desired cells, but also as a source for high quality iPS cells. That is, a non-pluripotent stem cell can be the starting point for the generation of high quality iPS cells by transfecting the non-pluripotent stem cell with one or more synthetic, modified RNAs encoding reprogramming factors, as described herein.

[00302] Stem cells are classified by their developmental potential as: (1) totipotent, meaning able to give rise to all embryonic and extraembryonic cell types; (2) pluripotent, meaning able to give rise to all embryonic cell types; (3) multipotent, meaning able to give rise to a subset of cell lineages, but all within a particular tissue, organ, or physiological system (for example, hematopoietic stem cells (HSC) can produce progeny that include HSC (self-renewal), blood cell restricted oligopotent progenitors and the cell types and elements (*e.g.*, platelets) that are normal components of the blood); (4) oligopotent, meaning able to give rise to a more restricted subset of cell lineages than multipotent stem cells; and (5) unipotent, meaning able to give rise to a single cell lineage (*e.g.*, spermatogenic stem cells).

[00303] Transfection with synthetic, modified RNAs directing the reprogramming of somatic, differentiated cells to pluripotency is specifically demonstrated herein. However, as also demonstrated herein, transfection with synthetic, modified RNAs can also be used to drive the differentiation, *i.e.*, decrease the developmental potential of stem cells other than iPS cells,

[00304] Stem cells of interest for producing cells with a desired phenotype or a reduced differentiation potential include embryonic cells of various types, exemplified by human embryonic stem (hES) cells, described by Thomson et al. (1998) Science 282:1145; embryonic stem cells from other primates, such as Rhesus stem cells (Thomson et al. (1995) Proc. Natl. Acad. Sci USA 92:7844); marmoset stem cells (Thomson et al. (1996) Biol. Reprod. 55:254); and human embryonic germ (hEG) cells (Shambloft et al., Proc. Natl. Acad. Sci. USA 95:13726, 1998). Also of interest are lineage committed stem cells, such as hematopoietic or pancreatic stem cells. In some embodiments, the host cell transfected with synthetic, modified RNA is a multipotent stem cell or progenitor cell. Examples of multipotent cells useful in methods provided herein include, but are not limited to, murine embryonic stem (ES-D3) cells, human umbilical vein endothelial (HuVEC) cells, human

umbilical artery smooth muscle (HuASMC) cells, human differentiated stem (HKB-Il) cells, and human mesenchymal stem (hMSC) cells. An additional stem cell type of interest for use with the compositions and methods described herein are cancer stem cells.

[00305] Adult stem cells are generally limited to differentiating into different cell types of their tissue of origin. However, if the starting stem cells are derived from the inner cell mass of the embryo, they can generate many cell types of the body derived from all three embryonic cell types: endoderm, mesoderm and ectoderm. Stem cells with this property are said to be pluripotent. Embryonic stem cells are one kind of pluripotent stem cell. Thus, pluripotent embryonic stem cells can be differentiated into many specific cell types, and that differentiation can be driven by the expression of polypeptides from synthetic, modified RNAs as described herein. Since the embryo is a potential source of all types of precursor cells, it is possible to differentiate embryonic stem cells into other lineages by providing the appropriate signals, such as the expression of proteins from synthetic, modified RNAs, to embryonic stem cells. Somatic stem cells also have major advantages, for example, using somatic stem cells allows a patient's own cells to be expanded in culture and then re-introduced into the patient. In addition and importantly, iPS cells generated from a patient provide a source of cells that can be expanded and re-introduced to the patient, before or after stimulation to differentiate to a desired lineage or phenotype. It is also contemplated that the compositions, methods and kits comprising the synthetic, modified RNAs described can be used to alter the developmental potential of a cancer stem cell, and thus render that cancer cell non-cancerous.

[00306] Cells derived from embryonic sources can include embryonic stem cells or stem cell lines obtained from a stem cell bank or other recognized depository institution. Other means of producing stem cell lines include the method of Chung et al (2006) which comprises taking a blastomere cell from an early stage embryo prior to formation of the blastocyst (at around the 8-cell stage). The technique corresponds to the pre-implantation genetic diagnosis technique routinely practiced in assisted reproduction clinics. The single blastomere cell is then co-cultured with established ES-cell lines and then separated from them to form fully competent ES cell lines.

[00307] Cells can also be derived from human umbilical cord blood cells (HUCBC), which are recognized as a rich source of hematopoietic and mesenchymal stem cells (Broxmeyer et al., 1992 Proc. Natl. Acad. Sci. USA 89:4109-4113). Cord blood cells are used as a source of transplantable stem and progenitor cells and as a source of marrow repopulating cells for the treatment of malignant diseases (*e.g.* acute lymphoid leukemia, acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome, and neuroblastoma) and non-malignant diseases such as Fanconi's anemia and aplastic anemia (Kohli-Kumar et al., 1993 Br. J. Haematol. 85:419-422; Wagner et al., 1992 Blood 79;1874-1881; Lu et al., 1996 Crit. Rev. Oncol. Hematol 22:61-78; Lu et al., 1995 Cell Transplantation 4:493-503). One advantage of HUCBC for use with the methods and compositions described herein is the immature immunity of these cells, which is very similar to fetal

cells, and thus significantly reduces the risk for rejection by the host (Taylor & Bryson, 1985 J. Immunol. 134:1493-1497).

[00308] In other embodiments of the aspects described herein, cancer stem cells are used with the synthetic, modified RNAs described herein, in order to, for example, differentiate or alter the phenotype of a cancer stem cell to a non-tumorigenic state. It has been recently discovered that stem-like cells are present in some human tumors and, while representing a small minority of the total cellular mass of the tumor, are the subpopulation of tumor cells responsible for growth of the tumor. In contrast to normal stem cells, "tumor stem cells" or "cancer stem cells" are defined as cells that can undergo self-renewal, as well as abnormal proliferation and differentiation to form a tumor. Functional features of tumor stem cells are that they are tumorigenic; they can give rise to additional tumorigenic cells by self-renewal; and they can give rise to non-tumorigenic tumor cells. As used herein, particularly in reference to an isolated cell or isolated cell population, the term "tumorigenic" refers to a cell derived from a tumor that is capable of forming a tumor, when dissociated and transplanted into a suitable animal model such as an immunocompromised mouse. The developmental origin of tumor stem cells can vary among different types of cancers. It is believed, without wishing to be bound or limited by theory, that tumor stem cells may arise either as a result of genetic damage that deregulates normal mechanisms of proliferation and differentiation of stem cells (Lapidot et al., Nature 367(6464): 645-8 (1994)), or by the dysregulated proliferation of populations of cells that acquire stem-like properties.

[00309] Tumors contain a distinct subset of cells that share the properties of normal stem cells, in that they proliferate extensively or indefinitely and that they efficiently give rise to additional solid tumor stem cells. Within an established tumor, most cells may have lost the ability to proliferate extensively and form new tumors, while tumor stem cells proliferate extensively and give rise to additional tumor stem cells as well as to other tumor cells that lack tumorigenic potential. An additional trait of tumor stem cells is their resistance to therapeutics, such as chemotherapy. It is the small fraction of tumor stem cells and their immediate daughter cell population that proliferates and ultimately proves fatal.

[00310] Examples of tumors from which samples containing cancer stem cells can be isolated from or enriched, for use with the compositions and methods described herein, include sarcomas and carcinomas such as, but not limited to: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, mesothelioma, Ewing's tumor, lymphangioendotheliosarcoma, synovioma, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor,

lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, astrocytic tumors (*e.g.*, diffuse, infiltrating gliomas, anaplastic astrocytoma, glioblastoma, gliosarcoma, pilocytic astrocytoma, pleomorphic xanthoastrocytoma), oligodendroglial tumors and mixed gliomas (*e.g.*, oligodendroglioma, anaplastic oligodendroglioma, oligoastrocytoma, anaplastic oligoastrocytoma), ependymal tumors (*e.g.*, ependymoma, anaplastic ependymoma, myxopapillary ependymoma, subependymoma), choroid plexus tumors, neuroepithelial tumors of uncertain origin (astroblastoma, chordoid glioma, gliomatosis cerebri), neuronal and mixed-neuronal-glial tumors (*e.g.*, ganglioglioma and gangliocytoma, desmoplastic infantile astrocytoma and ganglioglioma, dysembryoplastic neuroepithelial tumor, central neurocytoma, cerebellar liponeurocytoma, paraganglioglioma), pineal parenchymal tumors, embryonal tumors (medulloepithelioma, ependymoblastoma, medulloblastoma, primitive neuroectodemmal tumor, atypical teratoid/rhabdoid tumor), peripheral neuroblastic tumors, tumors of cranial and peripheral nerves (*e.g.*, schwannoma, neurinofibroma, perineurioma, malignant peripheral nerve sheath tumor), meningeal tumors (*e.g.*, meningeomas, mesenchymal, non-meningothelial tumors, haemangiopericytomas, melanocytic lesions), germ cell tumors, tumors of the sellar region (*e.g.*, craniopharyngioma, granular cell tumor of the neurohypophysis), hemangioblastoma, melanoma, and retinoblastoma. Additionally, the stem cell isolation methods of the invention are applicable to isolating stem cells from tissues other than characterized tumors (*e.g.*, from tissues of diseases such as the so called "stem cell pathologies").

[00311] Stem cells may be obtained from any mammalian species, *e.g.* human, primate, equine, bovine, porcine, canine, feline, rodent, *e.g.* mice, rats, hamster, etc. Embryonic stem cells are considered to be undifferentiated when they have not committed to a specific differentiation lineage. Such cells display morphological characteristics that distinguish them from differentiated cells of embryo or adult origin. Undifferentiated embryonic stem (ES) cells are easily recognized by those skilled in the art, and typically appear in the two dimensions of a microscopic view in colonies of cells with high nuclear/cytoplasmic ratios and prominent nucleoli.

[00312] In some embodiments, the stem cell is isolated. Most conventional methods to isolate a particular stem cell of interest involve positive and negative selection using markers of interest. For example, agents can be used to recognize stem cell markers, for instance labeled antibodies that recognize and bind to cell-surface markers or antigens on desired stem cells can be used to separate and isolate the desired stem cells using fluorescent activated cell sorting (FACS), panning methods, magnetic particle selection, particle sorter selection and other methods known to persons skilled in the art, including density separation (Xu et al. (2002) Circ. Res. 91:501; U.S. patent application Ser. No. 20030022367) and separation based on other physical properties (Doevendans et al. (2000) J. Mol. Cell. Cardiol. 32:839-851).

[00313] In those embodiments involving cancer stem cells, cancer stem cells can be identified using cell surface markers that also identify normal stem cells in the tissue of origin. As a non-limiting example, leukemic stem cells (LSCs) express the CD34 surface marker and lack the CD38

surface antigen, as is the case for normal (*i.e.*, non-leukemic) hematopoietic stem cells (Bonnet and Dick, 1997). Cancer stem cells identified by cell surface marker expression can be purified by methods known to one of skill in the art, such as fluorescence-activated cell sorting (FACS). Methods of isolating cancer stem cells can be found in United States Patent Application 20100003265.

[00314] Alternatively, genetic selection methods for isolating stem cells can be used, where a stem cell can be genetically engineered to express a reporter protein operatively linked to a tissue-specific promoter and/or a specific gene promoter, therefore the expression of the reporter can be used for positive selection methods to isolate and enrich the desired stem cell. For example, a fluorescent reporter protein can be expressed in the desired stem cell by genetic engineering methods to operatively link the marker protein to a promoter active in a desired stem cell (Klug et al. (1996) J. Clin. Invest. 98:216-224; U.S. Pat. No. 6,737,054). Other means of positive selection include drug selection, for instance as described by Klug et al., supra, involving enrichment of desired cells by density gradient centrifugation. Negative selection can be performed, selecting and removing cells with undesired markers or characteristics, for example fibroblast markers, epithelial cell markers etc.

[00315] Undifferentiated ES cells express genes that can be used as markers to detect the presence of undifferentiated cells, and whose polypeptide products can be used as markers for negative selection. For example, see U.S. application Ser. No. 2003/0224411 A1; Bhattacharya (2004) Blood 103(8):2956-64; and Thomson (1998), supra. Human ES cell lines express cell surface markers that characterize undifferentiated nonhuman primate ES and human EC cells, including stage-specific embryonic antigen (SSEA)-3, SSEA-4, TRA-I-60, TRA-1-81, and alkaline phosphatase. The globo-series glycolipid GL7, which carries the SSEA-4 epitope, is formed by the addition of sialic acid to the globo-series glycolipid Gb5, which carries the SSEA-3 epitope. Thus, GL7 reacts with antibodies to both SSEA-3 and SSEA-4. Undifferentiated human ES cell lines do not stain for SSEA-1, but differentiated cells stain strongly for SSEA-1. Methods for proliferating hES cells in the undifferentiated form are described in WO 99/20741, WO 01/51616, and WO 03/020920.

[00316] In some embodiments, the methods further provide for enrichment and isolation of stem cells. The stem cells are selected for a characteristic of interest. In some embodiments, a wide range of markers may be used for selection. One of skill in the art will be able to select markers appropriate for the desired cell type. The characteristics of interest include expression of particular markers of interest, for example specific subpopulations of stem cells and stem cell progenitors will express specific markers.

[00317] In some embodiments, the stem cells used with the compositions and methods described herein are expanded. The cells are optionally collected, separated, and further expanded generating larger populations of progenitor cells for use in making cells of a particular cell type or cells having a reduced differentiation potential.

Cell lines

[00318] In some embodiments, the cells used with the synthetic, modified RNAs described herein are cells of a cell line. In one such embodiment, the host cell is a mammalian cell line. In one such embodiment, the mammalian cell line is a human cell line.

[00319] Examples of human cell lines useful in methods provided herein include, but are not limited to, 293T (embryonic kidney), BT-549 (breast), DMS 114 (small cell lung), DU145 (prostate), HT-1080 (fibrosarcoma), HEK 293 (embryonic kidney), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), HL-60(TB) (leukemia), HS 578T (breast), HT-29 (colon adenocarcinoma), Jurkat (T lymphocyte), M14 (melanoma), MCF7 (mammary), MDA-MB-453 (mammary epithelial), PERC6® (E1-transformed embryonal retina), RXF 393 (renal), SF-268 (CNS), SF-295 (CNS), THP-1 (monocyte-derived macrophages), TK-10 (renal), U293 (kidney), UACC-257 (melanoma), and XF 498 (CNS).

[00320] Examples of rodent cell lines useful in methods provided herein include, but are not limited to, mouse Sertoli (TM4) cells, mouse mammary tumor (MMT) cells, rat hepatoma (HTC) cells, mouse myeloma (NS0) cells, murine hybridoma (Sp2/0) cells, mouse thymoma (EL4) cells, Chinese Hamster Ovary (CHO) cells and CHO cell derivatives, murine embryonic (NIH/3T3, 3T3 Ll) cells, rat myocardial (H9c2) cells, mouse myoblast (C2C12) cells, and mouse kidney (miMCD-3) cells.

[00321] Examples of non-human primate cell lines useful in methods provided herein include, but are not limited to, monkey kidney (CVI-76) cells, African green monkey kidney (VERO-76) cells, green monkey fibroblast (Cos-1) cells, and monkey kidney (CVI) cells transformed by SV40 (Cos-7). Additional mammalian cell lines are known to those of ordinary skill in the art and are catalogued at the American Type Culture Collection catalog (ATCC®, Mamassas, VA).

Other cell types

[00322] While mammalian cells are preferred, in some embodiments, the host cell transfected with a modified RNA is a plant cell, such as a tobacco plant cell.

[00323] In some embodiments, the transfected cell is a fungal cell, such as a cell from *Pichia pastoris*, a *Rhizopus* cell, or a *Aspergillus* cell.

[00324] In some embodiments, the transfected cell is an insect cell, such as SF9 or SF-21 cells from *Spodoptera frugiperda* or S2 cells from *Drosophila melanogaster*.

Cell Culture Methods

[00325] In general, cells useful with the methods described herein can be maintained and/or expanded in a culture medium that is available to and well-known in the art. Such media include, but are not limited to, Dulbecco's Modified Eagle's Medium® (DMEM), DMEM F12 Medium®, Eagle's Minimum Essential Medium®, F-12K Medium®, Iscove's Modified Dulbecco's Medium®, RPMI-

1640 Medium®, and serum-free medium for culture and expansion of progenitor cells SFEM®. Many media are also available as low-glucose formulations, with or without sodium.

[00326] Cells can be cultured in low-serum or serum-free "defined" culture medium. Serum-free medium used to culture cells is described in, for example, U.S. Pat. No. 7,015,037. Many cells have been grown in serum-free or low-serum medium. For example, the medium can be supplemented with one or more growth factors. Commonly used growth factors include, but are not limited to, bone morphogenic protein, basic fibroblast growth factor, platelet-derived growth factor and epidermal growth factor, Stem cell factor, and thrombopoietin. See, for example, U.S. Pat. Nos. 7,169,610; 7,109,032; 7,037,721; 6,617,161; 6,617,159; 6,372,210; 6,224,860; 6,037,174; 5,908,782; 5,766,951; 5,397,706; and 4,657,866 for teaching growing cells in serum-free medium.

[00327] Cells in culture can be maintained either in suspension or attached to a solid support, such as extracellular matrix components. Progenitor cells may require additional factors that encourage their attachment to a solid support, such as type I and type II collagen, chondroitin sulfate, fibronectin, "superfibronectin" and fibronectin-like polymers, gelatin, poly-D and poly-L-lysine, thrombospondin and vitronectin. Progenitor cells can also be cultured in low attachment flasks such as but not limited to Corning Low attachment plates.

[00328] In some embodiments, the host cells are suitable for growth in suspension cultures. Suspension-competent host cells are generally monodisperse or grow in loose aggregates without substantial aggregation. Suspension-competent host cells include cells that are suitable for suspension culture without adaptation or manipulation (*e.g.*, hematopoietic cells, lymphoid cells) and cells that have been made suspension-competent by modification or adaptation of attachment-dependent cells (*e.g.*, epithelial cells, fibroblasts).

[00329] In some embodiments, the host cell is an attachment dependent cell which is grown and maintained in adherent culture.

Altering Cellular Phenotypes and Developmental Potentials

[00330] The compositions and methods comprising the synthetic, modified RNAs described herein permit long-term, safe, and efficient alteration of cellular phenotypes or cellular developmental potentials, without the risk of permanent genomic alterations. Such compositions and methods are useful for a variety of applications, indications, and modalities, including, but not limited to, gene therapy, regenerative medicine, cancer therapies, tissue engineering, and drug screening.

[00331] Accordingly, provided herein are cells contacted with a synthetic, modified RNA molecule encoding a polypeptide, or a progeny cell of the contacted cell, where expression of the encoded polypeptide in the contacted cell alters a function or a developmental phenotype or developmental potential of the cell, and results in a reduced innate immune response relative to the cell contacted with a synthetic RNA molecule encoding the polypeptide not comprising any

modifications. In some embodiments, the developmental potential of the contacted cell is decreased. In some embodiments, the developmental potential of the contacted cell is increased. As such, the polypeptide encoded by the synthetic, modified RNA molecule can be a reprogramming factor, a differentiation factor, or a de-differentiation factor.

[00332] Also provided herein are cells comprising an exogenously introduced modified, synthetic RNA encoding a developmental potential altering factor. In some embodiments, the cell is a human cell. In some embodiments of these aspects, the cells or immediate precursor cell(s) have been subjected to at least 3 separate rounds of contacting with the modified, synthetic RNA encoding the developmental potential altering factor. In some such embodiments, the cells have a reduced expression of a Type I or Type II IFN relative to a cell subjected to at least 3 separate rounds of contacting with an exogenously introduced non-modified synthetic RNA encoding the developmental potential altering factor. In some such embodiments, the cell has a reduced expression of at least one IFN-signature gene relative to a human cell subjected to at least 3 separate rounds of contacting with an exogenously introduced non-modified synthetic RNA encoding the developmental potential altering factor. As described herein, the IFN-signature gene can be selected from the group consisting of IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20. The polypeptide encoded by the exogenous synthetic, modified RNA molecule can be a reprogramming factor, a differentiation factor, or a de-differentiation factor. The cell or its immediate precursor cell(s) can be derived from a somatic cell, a partially reprogrammed somatic cell, a pluripotent cell, a multipotent cell, a differentiated cell, or an embryonic cell.

[00333] As used herein, the term "developmental potential of a cell" refers to the total of all developmental cell fates or cell types that can be achieved by a cell upon differentiation. It should be understood that the developmental potential of a cell represents a spectrum: a terminally differentiated cell, *e.g.*, a cardiac myocyte, has essentially no developmental potential under natural conditions – that is, under normal circumstances, it cannot differentiate to another cell type; while at the other end of the spectrum, a totipotent embryonic stem cell has the potential to differentiate to or give rise to cells of every type in an organism, as well as the extra-embryonic structures. A cell with "parental developmental potential" refers to a cell having the developmental potential of the parent cell that gave rise to it.

[00334] The term "developmental potential of a cell" is relative. For example, where a stem cell undergoes differentiation to a more differentiated or specialized phenotype, the resulting cell has a reduced developmental potential relative to the stem cell that produced it. Unless specifically stated otherwise, the developmental potential of a cell is the potential it has assuming no further manipulation of its potential – that is, while it is acknowledged that the technology is available (as described herein) to artificially increase, decrease or otherwise alter the developmental potential of nearly any cell, to say that a cell has "reduced developmental potential" means that, without further

artificial manipulation to force the cell to a less differentiated phenotype, the cell can give rise to at least one fewer cell types than its immediate predecessor cell. That is, the cell resulting from a differentiation event has a reduced developmental potential despite the fact that it could possibly be manipulated to again become less differentiated. Thus, a cell with greater or higher developmental potential can differentiate into a greater variety of different cell types than a cell having a lower or decreased developmental potential.

[00335] Where, for example, a terminally- or only partially-differentiated cell is induced by artificial manipulation to become an induced pluripotent stem cell (an iPS cell), the resulting cell has increased developmental potential relative to the cell that produced it. As used herein, a "change" or "alteration" in the developmental potential of a cell occurs when the range of phenotypes to which a given cell can differentiate or give rise increases or decreases relative to the range naturally available to the cell prior to a differentiation, dedifferentiation or trans-differentiation event. By "increase" in this context is meant that there is at least additional one cell type or lineage to which a given cell can differentiate relative to the potential of the starting cell. By "decrease" in this context is meant that there is at least one fewer cell type or lineage to which the given cell can differentiate or give rise, relative to the potential of the starting cell.

[00336] Methods of manipulating the developmental potential of a cell, both to increase the potential and to decrease it, are described herein and others are known in the art. A "change" or "alteration" in the developmental potential of a cell can occur naturally, where, for example, a cell differentiates to a more specialized phenotype in its native environment *in vivo*. In various preferred aspects described herein, developmental potential or cell fate are directed by outside manipulation, and preferably by transfection with synthetic, modified RNA, as that term is defined herein. Thus, in one aspect, cells are contacted or transfected with synthetic, modified RNAs encoding one or more factors that re-direct or modify the phenotype of the cells.

[00337] Synthetic, modified RNAs as described herein can be made that direct the expression of essentially any gene product whose coding sequences can be cloned. The expression of the gene product from synthetic, modified RNA introduced to a cell that does not normally express that gene product necessarily results in a change in the phenotype of the cell whether or not it changes the differentiation status or differentiation potential of the cell. Simply put, the new phenotype is the cell's expression of the new gene product. Thus, in one aspect, encompassed herein is the expression of a protein from a synthetic, modified RNA introduced to a cell. Expression that does not necessarily change the differentiation status of the cell can nonetheless be useful in such embodiments, for example, where one wishes to correct or replace a defective function in a cell, due to a genetic defect or polymorphism, or in embodiments to target a cell to a particular location, *e.g.*, by expressing a receptor or where one wishes to induce cell death in *e.g.*, a tumor by expressing a death receptor, a death ligand, a cell cycle inhibitor etc.

[00338] In other aspects, the synthetic, modified RNAs described herein are well suited for directing the expression of any gene sequence, but are particularly well suited for modifying the differentiation status or the developmental potential of a cell, and for doing so without permanent change to the genome of the cell. This is true in part because reprogramming, differentiation and transdifferentiation each require relatively prolonged expression of one or more polypeptide factors in a target cell. Non-modified RNA is recognized as foreign by the cell's innate immune defenses against viral and bacterial RNA. If the cell transfected with non-modified RNA is not induced to undergo apoptosis or to otherwise shut down protein synthesis by a first transfection event, it will likely do so upon a subsequent transfection event with unmodified RNA.

Reprogramming

[00339] The production of cells having an increased developmental potential (*e.g.*, iPS cells) is generally achieved by the introduction of nucleic acid sequences, specifically DNA, encoding stem cell-associated genes into an adult, somatic cell. Historically, these nucleic acids have been introduced using viral vectors and the expression of the gene products results in cells that are morphologically, biochemically, and functionally similar to pluripotent stem cells (*e.g.*, embryonic stem cells). This process of altering a cell phenotype from a somatic cell phenotype to a pluripotent stem cell phenotype is termed "reprogramming." In the reprogramming methods described herein, the reprogramming is achieved by repeated transfection with synthetic, modified RNAs encoding the necessary reprogramming factors. The repeated transfection provides prolonged expression of the factors encoded by the synthetic, modified RNAs necessary to shift the developmental potential of the cell.

[00340] Accordingly, provided herein are pluripotent cells that are not embryonic stem cells, and which were not induced by viral expression of one or more reprogramming factors, and which when subjected to an unsupervised hierarchical cluster analysis, cluster more closely to embryonic stem cells than do pluripotent cells induced by viral expression of one or more reprogramming factors, exogenous protein introduction of one or more reprogramming factors, small molecule mediated expression or induction of one or more reprogramming factors, or any combination thereof. In some aspects, provided herein are pluripotent cells that are not embryonic stem cells, and which were not induced by viral expression of one or more reprogramming factors. In such aspects, the pluripotent cell subjected to an unsupervised hierarchical cluster analysis clusters more closely to a human embryonic stem cell than does a pluripotent cell induced by viral expression of one or more reprogramming factors. The pluripotent cell is generated from a precursor somatic cell, such as a precursor human somatic cell. The pluripotent cell or its immediate precursor cell(s) can also be derived from a somatic cell, partially reprogrammed somatic cell, a pluripotent cell, a multipotent cell, a differentiated cell, or an embryonic cell.

[00341] Reprogramming to generate pluripotent cells, as described herein, can be achieved by introducing a one or more synthetic, modified RNAs encoding stem cell-associated genes including,

for example Oct-4 (also known as Oct-3/4 or Pouf51) (SEQ ID NO: 788), Sox1, Sox2 (SEQ ID NO: 941 or SEQ ID NO: 1501), Sox3, Sox 15, Sox 18, NANOG, Klf1, Klf2, Klf4 (SEQ ID NO: 501), Klf5, NR5A2, c-Myc (SEQ ID NO: 636), l-Myc, n-Myc, Rem2, Tert, LIN28 (SEQ ID NO: 524), and Sall4. Accordingly, in some embodiments, the reprogramming factor is selected from the group consisting of: OCT4, SOX1, SOX 2, SOX 3, SOX15, SOX 18, NANOG, KLF1, KLF 2, KLF 4, KLF 5, NR5A2, c-MYC, l- MYC, n- MYC, REM2, TERT, and LIN28. In general, successful reprogramming is accomplished by introducing at least Oct-4, a member of the Sox family, a member of the Klf family, and a member of the Myc family to a somatic cell. In some embodiments, LIN28 is also introduced. The generation of iPS cells using transfection of the synthetic, modified RNAs described herein, also referred to herein as "RiPS," from a variety of starting cell types, including an adult somatic cell, is demonstrated in the Examples herein. The generation of reprogrammed cells using the compositions and methods described herein preferably causes the induction of endogenous stem-cell associated genes, such as SOX2, REX1, DNMT3B, TRA-1-60, TRA-1-81, SSEA3, SSEA4, OCT4, and NANOG. In some embodiments, at least two endogenous stem-cell-associated genes are induced. Preferably, the endogenous expression is at a level comparable to an embryonic stem cell, such as an embryonic stem cell cultured within the same laboratory.

[00342] The methods to reprogram cells using the synthetic, modified RNAs described herein can involve repeated contacting of the cells, such as somatic cells, in order to permit sufficient expression of the encoded reprogramming factors to maintain a stable change in the developmental potential of the cells, or progeny cells thereof, being contacted. Such methods can involve repeated transfections, such as for example, at least two, at least five, at least 6 ,at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 25, at least 30, or more transfections. In other words, the methods comprise repeating transfection using the synthetic, modified RNAs until a desired phenotype of the cell or population of cells is achieved. In some embodiments, the methods further comprise contacting with or introducing the reprogramming factors to the cells under low-oxygen conditions.

[00343] The efficiency of reprogramming (*i.e.*, the number of reprogrammed cells) can be enhanced by the addition of various small molecules as shown by Shi, Y., et al (2008) *Cell-Stem Cell* 2:525-528, Huangfu, D., et al (2008) *Nature Biotechnology* 26(7):795-797, and Marson, A., et al (2008) *Cell-Stem Cell* 3:132-135. It is contemplated that the methods described herein can also be used in combination with a single small molecule (or a combination of small molecules) that enhances the efficiency of induced pluripotent stem cell production or replaces one or more reprogramming factors during the reprogramming process. Some non-limiting examples of agents that enhance reprogramming efficiency include soluble Wnt, Wnt conditioned media, BIX-01294 (a G9a histone methyltransferase), PD0325901 (a MEK inhibitor), DNA methyltransferase inhibitors, histone deacetylase (HDAC) inhibitors, valproic acid, 5'-azacytidine, dexamethasone, suberoylanilide, hydroxamic acid (SAHA), and trichostatin

(TSA), among others.

[00344] In some embodiments of the aspects described herein, an inhibitor of p53 can be used to reduce the stress response during a reprogramming regimen to direct the cell fate away from an apoptotic stimulus and towards reprogramming. Thus, treatment with a p53 inhibitor can enhance reprogramming in a population of cells. In one such embodiment, the inhibitor of p53 comprises an siRNA directed against p53 that is administered or expressed in the reprogramming cell. In another embodiment, a small molecule inhibitor of p53 (*e.g.*, pifithrin-α) is administered to cells during the reprogramming process. In one embodiment, a modified RNA encoding Bcl2 is administered to the cells prior to, or in conjunction with, a modified RNA composition encoding at least one reprogramming factor to prevent apoptosis of cells during the process of reprogramming.

[00345] To confirm the induction of pluripotent stem cells, isolated clones can be tested for the expression of an endogenous stem cell marker. Such expression identifies the cells as induced pluripotent stem cells. Stem cell markers can be selected from the non-limiting group including SSEA1, CD9, Nanog, Fbx15, Ecat1, Esg1, Eras, Gdf3, Fgf4, Cripto, Dax1, Zpf296, Slc2a3, Rex1, Utf1, and Nat1. Methods for detecting the expression of such markers can include, for example, RT-PCR and immunological methods that detect the presence of the encoded polypeptides. Further evidence of reprogramming is shown by a reduction in or the loss of lamin A/C protein expression. Alternatively, reprogramming is detected by measuring an increase in acetylation, such as increased acetylation of H3 and H4 within the promoter of Oct4, or by measuring a decrease in methylation, for example, by measuring the demethylation of lysine 9 of histone 3. In each of these cases, reprogramming is measured relative to a control cell. In other embodiments, reprogramming is assayed by any other method that detects chromatin remodeling leading to the activation of an embryonic stem cell marker, such as Oct4.

[00346] The pluripotent stem cell character of the isolated cells can be confirmed by any of a number of tests evaluating the expression of ES markers and the ability to differentiate to cells of each of the three germ layers. As one example, teratoma formation in nude mice can be used to evaluate the pluripotent character of the isolated clones. The cells are introduced to nude mice and histology and/or immunohistochemistry is performed on a tumor arising from the cells. The growth of a tumor comprising cells from all three germ layers further indicates that the cells are pluripotent stem cells.

[00347] The pluripotent cells generated using the compositions and methods comprising the synthetic, modified RNAs described herein cluster more closely to a human embryonic stem cell than do pluripotent cells induced by viral expression of one or more reprogramming factors, when subjected to an unsupervised hierarchical analysis, *i.e.*, the pluripotent cells have a phenotype closer to a embryonic stem cell phenotype than do pluripotent cells induced by viral expression of one or more reprogramming factors. In some embodiments, the unsupervised hierarchical cluster analysis is performed using a Euclidean distance with average linkage method in which the similarity metric for comparison between different cells is indicated on the height of cluster dendrogram. The unsupervised

hierarchical cluster analysis can be performed on any data set available to a skilled artisan, such as gene expression data, protein expression data, DNA methylation data, histone modification data, and microRNA data.

[00348] Clustering, including, "unsupervised clustering analysis" or "unsupervised cluster analysis" refers to methods used in multivariate analysis to divide up objects into similar groups, or, in some embodiments, groups whose members are all close to one another on various dimensions being measured in the various objects. A key component of the analysis is repeated calculation of distance measures between objects, and between clusters once objects begin to be grouped into clusters. The outcome is typically represented graphically as a dendrogram. Hierarchical cluster analysis can be performed using any of a variety of unbiased computational methods, algorithms and software programs known to one of skill in the art that identify clusters or natural data structures from large data sets, such as, for example, gene expression data sets. Such methods include, but are not limited to, bottom-up hierarchical clustering, K-means clustering Affinity Propagation, non-Negative Matrix Factorization, spectral clustering, Self-Organizing Map (SOM) algorithms, and the like. In some embodiments of the aspects described herein, one SOM-based method for use in unsupervised hierarchical clustering analysis of cells contacted with the synthetic, modified RNAs described herein is the Automatic clustering using density-equalized SOM Ensembles (AUTOsome) method as described in A.M. Newman and J.B. Cooper (2010, Cell Stem Cell, 7:258-262) and A.M. Newman and J.B. Cooper (2010, BMC Bioinformatics 2010, 11:117).

[00349] Accordingly, also provided herein are compositions for generating such pluripotent cells, comprising at least one synthetic, modified RNA encoding a reprogramming factor, and cell growth media. The synthetic, modified RNAs can comprise any modification for reducing the innate immune response, as described herein, such as a 5' cap, a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof. In preferred embodiments, the synthetic, modified RNAs comprise at least two nucleoside modifications, preferably 5-methylcytidine (5mC) and pseudouridine.

[00350] In some embodiments, the compositions permit an efficiency of pluripotent cell generation from a starting population of cells, such as somatic cells, of at least 1%. In some embodiments, the efficiency of pluripotent cell generation is at least 1.1%, at least 1.2%, at least 1.3%, at least 1.4%, at least 1.5%, at least 1.6%, at least 1.7%, at least 1.8%, at least 1.9%, at least 2.0%, at least 2.1%, at least 2.2%, at least 2.3%, at least 2.4%, at least 2.5%, at least 2.6%, at least 2.7%, at least 2.8%, at least 2.9%, at least 3.0%, at least 3.1%, at least 3.2%, at least 3.3%, at least 3.4%, at least 3.5%, at least 3.6%, at least 3.7%, at least 3.8%, at least 3.9%, at least 4.0%, at least 4.1%, at least 4.2%, at least 4.3%, at least 4.4%, at least 4.5%, at least 4.6%, at least 4.7%, at least 4.8%, at least 4.9%, at least 5.0%, 5.1%, at least 5.2%, at least 5.3%, at least 5.4%, at least 5.5%, at least 5.6%, at least 5.7%, at least 5.8%, at least 5.9%, at least 6.0%, 6.1%, at least 6.2%, at least 6.3%, at least

6.4%, at least 6.5%, at least 6.6%, at least 6.7%, at least 6.8%, at least 6.9%, at least 7.0%, 7.1%, at least 8.2%, at least 8.3%, at least 8.4%, at least 8.5%, at least 8.6%, at least 8.7%, at least 8.8%, at least 8.9%, at least 9.0%, 9.1%, at least 9.2%, at least 9.3%, at least 9.4%, at least 9.5%, at least 1.6%, at least 9.7%, at least 9.8%, at least 9.9%, at least 10.0%, or more.

[00351] In some embodiments, the compositions permit a rate of pluripotent cell generation from a starting population of cells, such as somatic cells of less than 25 days, less than 24 days, less than 23 days, less than 22 days, less than 21 days, 20 days, less than 19 days, less than 18 days, less than 17 days, less than 16 days, less than 15 days, less than 14 days, and greater than 7 days.

[00352] The reprogramming factor(s) for use in the compositions, methods, and kits for reprogramming cells described herein is selected from the group consisting of: OCT4 (SEQ ID NO: 788), SOX1, SOX 2 (SEQ ID NO: 941 or SEQ ID NO: 1501), SOX 3, SOX15, SOX 18, NANOG, KLF1, KLF 2, KLF 4 (SEQ ID NO: 501), KLF 5, NR5A2, c-MYC (SEQ ID NO: 636), l- MYC, n-MYC, REM2, TERT, and LIN28 (SEQ ID NO: 524). In some embodiments, the compositions comprise at least 4 synthetic, modified RNAs encoding at least 4 different reprogramming factors. In some such embodiments, the at least 4 different reprogramming factors encoded by the at least 4 modified synthetic RNAs comprise OCT4, SOX2, KLF4, and c-MYC. The compositions can further comprise a modified synthetic RNA encoding a LIN28 reprogramming factor. In some embodiments, the composition does not comprise a modified, synthetic RNA encoding the reprogramming factor c-MYC.

Transdifferentiation

[00353] Transdifferentiation refers to a process by which the phenotype of a cell can be switched to that of another cell type, without the formation of a pluripotent intermediate cell. Thus, the methods do not require that the cell first be de-differentiated (or reprogrammed) and then differentiated to another cell type; rather the cell type is merely "switched" from one cell type to another without first forming a less differentiated phenotype. Thus, “transdifferentiation” refers to the capacity of differentiated cells of one type to lose identifying characteristics and to change their phenotype to that of other fully differentiated cells.

[00354] Transdifferentiation can be achieved by introducing into a cell a synthetic, modified RNA composition that permits expression of a cell-type specific differentiation factor. For example, to transdifferentiate a cell to a myogenic lineage one can express MyoD using a modified RNA as described herein. While the introduction of a single differentiation factor can be enough to transdifferentiate a cell, it is also contemplated herein that a plurality of different differentiation factors are introduced to the cell during the transdifferentiation regime. Alternatively, synthetic, modified RNAs that inhibit expression of cell-type specific polypeptides of the original cell-type can also be introduced to the cell, in effect "turning off" the original phenotype of the cell. In one embodiment, modified RNAs that express a desired cell-type specific polypeptide to turn on a desired phenotype are used in combination with modified RNA interference molecules used to turn off the

existing cell phenotype, in order to cause transdifferentiation of the cell from one phenotype to another.

[00355] Transdifferentiation can be useful in tissue engineering at *e.g.*, an injury or disease site. In one embodiment, transdifferentiation is performed *in vivo* at the site of injury or disease. In another embodiment, an organ or tissue can be transdifferentiated/ regenerated *in vitro*, and then introduced back into the body.

Differentiation

[00356] Differentiation is the process by which an unspecialized ("uncommitted") or less specialized cell acquires the features of a specialized cell (*e.g.*, a terminally differentiated cell) such as, for example, a cardiomyocyte, a nerve cell or a skeletal muscle cell. A differentiated or differentiation-induced cell is one that has taken on a more specialized ("committed") position within the lineage of a cell (*e.g.*, reduced differentiation potential). The term "committed", when applied to the process of differentiation, refers to a cell that has proceeded in the differentiation pathway to a point where, under normal circumstances, it will continue to differentiate into a specific cell type or subset of cell types, and cannot, under normal circumstances, differentiate into a different cell type or revert to a less differentiated cell type. De-differentiation refers to the process by which a cell reverts to a less specialized (or committed) position within the lineage of a cell (*i.e.*, increased developmental potential). As used herein, the lineage of a cell defines the heredity or fate of the cell, *i.e.*, which cells it came from and what cells it can give rise to. The lineage of a cell places the cell within a hereditary scheme of development and differentiation. A lineage-specific marker refers to a characteristic specifically associated with the phenotype of cells of a lineage of interest and can be used to assess the differentiation of an uncommitted cell to the lineage of interest.

[00357] Cells that are differentiated using the compositions and methods comprising synthetic, modified RNAs, as described herein, can be differentiated into any cell type or lineage known to one of skill in the art. Such cells can be of a lineage selected from an ectodermal lineage, a mesodermal lineage, or an endodermal lineage. Exemplary ectodermal lineage cells include, but are not limited to, cells of the epidermis (skin cells, melanocytes), and cells of the neuronal lineage. Exemplary mesodermal lineage cells include, but are not limited to, cells of the circulatory system (cardiac cells and blood vessel cells), cells of the connective tissue, bone cells, dermal cells, myocytes (smooth and skeletal), certain cells of the urinary system, such as kidney cells, splenic cells, mesothelial cells (cells of the peritoneum, pleura, and pericardium), non-germ cells of the reproductive system, and hematopoietic lineage cells. Exemplary endodermal lineage cells include, but are not limited to, cells of the gastrointestinal system, cells of the respiratory tract, cells of the endocrine glands, cells of the auditory system, and certain cells of the urinary system, such as the bladder and parts of the urethra.

[00358] Accordingly, compositions and methods described herein include a method for programming or directing the differentiation of cells (*e.g.*, stem cells) comprising contacting the cells desired to be differentiated with a synthetic, modified RNA or synthetic, modified RNA composition.

The cells can be transfected a plurality of times until the desired differentiated phenotype is achieved, as measured by *e.g.*, a gene expression array of cell-type specific markers, Western blotting, cell function assays etc. A selection compound may be added to the mixture, but is not required.

[00359] Typically, the synthetic, modified RNA composition transfected into the cells to promote their differentiation encodes a cell-type specific differentiation factor or factors. For example, to differentiate a cell to a neuronal cell phenotype, a synthetic, modified RNA encoding at least one neuronal differentiation factor, for example Ascl1, Brn2, Myt1l, or a combination thereof is transfected into the cell. To promote differentiation to a myogenic phenotype, a synthetic, modified RNA such as one encoding MyoD can be transfected into a cell. To differentiate a cell to a macrophage phenotype, a macrophage factor such as *e.g.*, CEBP-alpha or PU.1 is transfected into the cell. In one embodiment, a modified RNA that encodes Ngn3, Pdx1, MAFA, or any combination thereof can be used to differentiate cells to a pancreatic beta cell phenotype. A synthetic, modified RNA encoding PRDM16 can be applied to Myf5-expressing progenitors to induce differentiation into brown fat cells. Oligodendrocytes may be specified from neural precursors using a synthetic, modified RNA encoding Ascl1. It has been reported that hepatocyte differentiation requires the transcription factor HNF-4α. (Li et al., Genes Dev. 14:464, 2000). A synthetic, modified RNA can be applied to a cell, such as a stem cell or induced pluripotent stem cell generated using the compositions described herein, that inhibit or suppress one or more component of the wnt/β-catenin pathway to become a cardiovascular progenitor cell. These examples are not meant to be limiting and essentially any cell-type specific factor or differentiation factor known in the art can be expressed in a cell using a synthetic, modified RNA or synthetic, modified RNA composition as described herein. **Table 1** provides a non-limiting list of exemplary transcription factors and mRNA sequence identifiers that can be used to alter the developmental potential or phenotype of a cell.

[00360] In other embodiments, cells with higher or increased developmental potential, *e.g.*, pluripotent cells, multipotent cells, etc., can be induced to differentiate by manipulating their external environment. For example, cells can be maintained under culture conditions that induce differentiation of the cells to a desired lineage. As but one example, in some embodiments, cells with higher or increased developmental potential, generated using the compositions and methods comprising synthetic, modified RNAs described herein, can be differentiated into islet-like cells for administration to a patient in need thereof, for example, a patient having or at risk for diabetes. In such embodiments, islet-like cells, which includes insulin-producing cells and glucagon-producing cells, can be differentiated using any of the methods described in US Patent Publication No.: 20100240130. For example, cells can be differentiated whereby the first culturing step takes place in the presence of an Activin, the next culturing step utilizes a suspension culture that takes place in the presence of a noggin, an FGF-2, and an EGF, and a final culturing step in which the cells are cultured with nicotinamide. In certain embodiments, sodium butyrate can be included in the culture medium. In other embodiments, pluripotent cells can be differentiated into islet-like cells by directed

differentiation. In certain embodiments, expression of additional genes at the site of islet-like cell administration, using the compositions and methods described herein, can facilitate adoption of the functional β-islet cell phenotype, enhance the beneficial effect of the administered cells, and/or increase proliferation and/or activity of host cells neighboring the treatment site.

[00361] In other embodiments, cells with higher or increased developmental potential, generated using the compositions and methods comprising synthetic, modified RNAs described herein, can be differentiated, for example, into neuronal cells, such as oligodendrocytes, for example, for treatment of spinal cord injuries. In such embodiments, pluripotent cells can be differentiated using any of the compositions or methods found in US Patent Publication No.: 20090232779 or US Patent Publication No.: 20090305405. For example, cells can be differentiated to neural or glial lineages, using medium including any of the following factors in an effective combination: Brain derived neurotrophic factor (BDNF), neutrotrophin-3 (NT-3), NT-4, epidermal growth factor (EGF), ciliary neurotrophic factor (CNTF), nerve growth factor (NGF), retinoic acid (RA), sonic hedgehog, FGF-8, ascorbic acid, forskolin, fetal bovine serum (FBS), and bone morphogenic proteins (BMPs).

[00362] In other exemplary embodiments, cells with higher or increased developmental potential generated using the compositions and methods comprising synthetic, modified RNAs described herein can be differentiated into heptaocyte-like cells for treatment of liver diseases, such as cirrhosis. For example, cells can be differentiated to hepatocyte-like cells, using medium including any of the following factors in an effective combination or sequence: a hepatocyte supportive extracellular matrix, such as collagen or Matrigel; suitable differentiation agents, such as various isomers of butyrate and their analogs, exemplified by n-butyrate; a hepatocyte maturation factor, such as an organic solvent like dimethyl sulfoxide (DMSO); a maturation cofactor such as retinoic acid; a cytokine or hormone such as a glucocorticoid, epidermal growth factor (EGF), insulin, transforming growth factors (TGF-α and TGF-β), fibroblast growth factors (FGF), heparin, hepatocyte growth factors (HGF), interleukins (IL-1 and IL-6), insulin-like growth factors (IGF-I and IGF-II), and heparin-binding growth factors (HBGF-1).

[00363] The success of a differentiation program can be monitored by any of a number of criteria, including characterization of morphological features, detection or quantitation of expressed cell markers and enzymatic activity, and determination of the functional properties of the desired end cell types *in vitro* or *in vivo*. The level of mRNA corresponding to a marker can be determined both by in situ and by *in vitro* formats. The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. Protein markers can be measured *e.g.*, by immunohistochemical techniques or the morphology of the cell can be monitored. Biochemical approaches, *e.g.*, the ability of the

differentiated cell to respond to a cell-type specific stimulus can also be monitored. An increase in the expression of a cell specific marker may be by about 5%, 10%, 25%, 50%, 75% or 100%. In one embodiment, the synthetic, modified RNA composition can direct cell fate towards different germ layers without definitively specifying a terminally differentiated cell type. For example, a synthetic, modified RNA encoding Sox17 or GATA6 can be used for definitive endodermal specification from pluripotent cells, such as an iPS or embryonic stem cell. Similarly, a synthetic, modified RNA encoding T (Brachyury) can be used for specification of mesoderm. For example, markers for neural cells include, but are not limited to: β-tubulin III or neurofilament, which are characteristic of neurons, glial fibrillary acidic protein (GFAP), present in astrocytes; galactocerebroside (GalC) or myelin basic protein (MBP), characteristic of oligodendrocytes; nestin, characteristic of neural precursors and other cells, and A2B5 and NCAM, characteristic of glial progenitors and neural progenitors, respectively. Similarly, an adipocyte can be detected by assaying for Oil-Red-O staining or acetylated LDL uptake. Cardiomyocytes can be detected by assaying for the expression of one or more cardiomyocyte specific markers, such as cardiotroponin I, Mef2c, connexin43, Nkx2.5, GATA-4, sarcomeric actinin, cariotroponin T and TBX5, and sarcomeric actinin, α-cardiac myosin heavy chain, actin, or ventricular myosin light chain 2 (MLC-2v). For skeletal muscle, markers include myoD, myogenin, and myf-5. Markers of interest for identifying liver cells include α-fetoprotein (liver progenitors); albumin, α_1-antitrypsin, glucose-6-phosphatase, cytochrome p450 activity, transferrin, asialoglycoprotein receptor, and glycogen storage (hepatocytes); CK7, CK19, and γ-glutamyl transferase (bile epithelium). The presence of endothelial cells can be detected by assaying the presence of an endothelial cell specific marker, such as CD31+, PECAM (platelet endothelial cell adhesion molecule), Flk-1, tie-1, tie-2, vascular endothelial (VE) cadherin, MECA-32, and MEC-14.7. For pancreatic cells, pdx and insulin secretion can be used for determination of differentiation. The level of expression can be measured in a number of ways, including, but not limited to: measuring the mRNA encoded by the markers; measuring the amount of protein encoded by the markers; or measuring the activity of the protein encoded by the markers.

[00364] In some embodiments, differentiation is detected by measuring an alteration in the morphology or biological function or activity of a differentiated cell. An alteration in biological function may be assayed, for example, by measuring an increase in acetylated LDL uptake in a reprogrammed adipocyte. For example, GABA-secreting neurons can be identified by production of glutamic acid decarboxylase or GABA. Dopaminergic neurons can be identified by production of dopa decarboxylase, dopamine, or tyrosine hydroxylase. Also, for example, differentiated hepatocyte lineage cells differentiated can be identified by α_1-antitrypsin (AAT) synthesis, albumin synthesis, evidence of glycogen storage, evidence of cytochrome p450 activity, and evidence of glucose-6-phosphatase activity. Other methods for assaying cell morphology and function are known in the art and are described in the Examples.

[00365] In some embodiments, the cells of the compositions and methods described herein are further cultured in the presence of cell specific growth factors, such as angiogenin, bone morphogenic protein-1, bone morphogenic protein-2, bone morphogenic protein-3, bone morphogenic protein-4, bone morphogenic protein-5, bone morphogenic protein-6, bone morphogenic protein-7, bone morphogenic protein-8, bone morphogenic protein-9, bone morphogenic protein-10, bone morphogenic protein-11, bone morphogenic protein-12, bone morphogenic protein-13, bone morphogenic protein-14, bone morphogenic protein-15, bone morphogenic protein receptor IA, bone morphogenic protein receptor IB, brain derived neurotrophic factor, ciliary neutrophic factor, ciliary neutrophic factor receptor-alpha, cytokine-induced neutrophil chemotactic factor 1, cytokine-induced neutrophil, chemotactic factor 2-alpha, cytokine-induced neutrophil chemotactic factor 2-beta, beta-endothelial cell growth factor, endothelia 1, epidermal growth factor, epithelial-derived neutrophil attractant, fibroblast growth factor 4, fibroblast growth factor 5, fibroblast growth factor 6 fibroblast growth factor 7, fibroblast growth factor 8, fibroblast growth factor b, fibroblast growth factor c, fibroblast growth factor 9, fibroblast growth factor 10, fibroblast growth factor acidic, fibroblast growth factor basic, glial cell line-derived neutrophil factor receptor-alpha-1, glial cell line-derived neutrophil factor receptor-alpha-2, growth related protein, growth related protein-alpha, growth related protein-beta, growth related protein-gamma, heparin binding epidermal growth factor, hepatocyte growth factor, hepatocyte growth factor receptor, insulin-like growth factor I, insulin-like growth factor receptor, insulin-like growth factor II, insulin-like growth factor binding protein, keratinocyte growth factor, leukemia inhibitory factor, leukemia inhibitory factor receptor-alpha, nerve growth factor, nerve growth factor receptor, neurotrophin-3, neurotrophin-4, placenta growth factor, placenta growth factor 2, platelet-derived endothelial cell growth factor, platelet derived growth factor, platelet derived growth factor A chain, platelet derived growth factor AA, platelet derived growth factor AB, platelet derived growth factor B chain, platelet derived growth factor BB, platelet derived growth factor receptor-alpha, platelet derived growth factor receptor-beta, pre-B cell growth stimulating factor, stem cell factor, stem cell factor receptor, transforming growth factor-alpha, transforming growth factor-beta, transforming growth factor-beta-1, transforming growth factor-beta-1-2, transforming growth factor-beta-2, transforming growth factor-beta-3, transforming growth factor-beta-5, latent transforming growth factor-beta-1, transforming growth factor-beta-binding protein I, transforming growth factor-beta-binding protein II, transforming growth factor-beta-binding protein III, tumor necrosis factor receptor type I, tumor necrosis factor receptor type II, urokinase-type plasminogen activator receptor, vascular endothelial growth factor, and chimeric proteins and biologically or immunologically active fragments thereof. Such factors can also be injected or otherwise administered directly into an animal system for *in vivo* integration.

Cell modifications

Homing Moieties and Cell-Surface Receptors

[00366] In some aspects and embodiments of the aspects described herein, a synthetic, modified RNA can be used to express a ligand or ligand receptor on the surface of a cell (*e.g.*, a homing moiety). A ligand or ligand receptor moiety attached to a cell surface permits the cell to have a desired biological interaction with a tissue or an agent *in vivo*. A ligand can be an antibody, an antibody fragment, an aptamer, a peptide, a vitamin, a carbohydrate, a protein or polypeptide, a receptor, e.g., cell-surafce receptor, an adhesion molecule, a glycoprotein, a sugar residue, a therapeutic agent, a drug, a glycosaminoglycan, or any combination thereof. For example, a ligand can be an antibody that recognizes a cancer-cell specific antigen, rendering the cell capable of preferentially interacting with tumor cells to permit tumor-specific localization of a modified cell. A ligand can confer the ability of a cell composition to accumulate in a tissue to be treated, since a preferred ligand is capable of interacting with a target molecule on the external face of a tissue to be treated. Ligands having limited cross-reactivity to other tissues are generally preferred.

[00367] In some cases, a ligand can act as a homing moiety which permits the cell to target to a specific tissue or interact with a specific ligand. Such homing moieties can include, for example, any member of a specific binding pair, antibodies, monoclonal antibodies, or derivatives or analogs thereof, including without limitation: Fv fragments, single chain Fv (scFv) fragments, Fab' fragments, $F(ab')_2$ fragments, single domain antibodies, camelized antibodies and antibody fragments, humanized antibodies and antibody fragments, and multivalent versions of the foregoing; multivalent binding reagents including without limitation: monospecific or bispecific antibodies, such as disulfide stabilized Fv fragments, scFv tandems ($(scFv)_2$ fragments), diabodies, tribodies or tetrabodies, which typically are covalently linked or otherwise stabilized (*i.e.*, leucine zipper or helix stabilized) scFv fragments; and other homing moieties include for example, aptamers, receptors, and fusion proteins.

[00368] In some embodiments, the homing moiety is a surface-bound antibody, which can permit tuning of cell targeting specificity. This is especially useful since highly specific antibodies can be raised against an epitope of interest for the desired targeting site. In one embodiment, multiple antibodies are expressed on the surface of a cell, and each antibody can have a different specificity for a desired target. Such approaches can increase the avidity and specificity of homing interactions.

[00369] A skilled artisan can select any homing moiety based on the desired localization or function of the cell, for example an estrogen receptor ligand, such as tamoxifen, can target cells to estrogen-dependent breast cancer cells that have an increased number of estrogen receptors on the cell surface. Other non-limiting examples of ligand/receptor interactions include CCR1 (*e.g.*, for treatment of inflamed joint tissues or brain in rheumatoid arthritis, and/or multiple sclerosis), CCR7, CCR8 (*e.g.*, targeting to lymph node tissue), CCR6, CCR9,CCR10 (*e.g.*, to target to intestinal tissue), CCR4, CCR10 (*e.g.*, for targeting to skin), CXCR4 (*e.g.*, for general enhanced transmigration), HCELL (*e.g.*, for treatment of inflammation and inflammatory disorders, bone marrow), Alpha4beta7 (*e.g.*, for intestinal mucosa targeting), VLA-4 / VCAM-1 (*e.g.*, targeting to endothelium). In general, any receptor involved in targeting (*e.g.*, cancer metastasis) can be harnessed for use in the methods and

compositions described herein. **Table 2** and **Table 3** provide non-limiting examples of CD ("cluster of differentiation") molecules and other cell-surface/membrane bound molecules and receptors that can be expressed using the synthetic, modified RNA compositions and methods described herein for targeting and homing to cells of interest, or for changing the phenotype of a cell.

Mediators of cell death

[00370] In one embodiment, a synthetic, modified RNA composition can be used to induce apoptosis in a cell (*e.g.*, a cancer cell) by increasing the expression of a death receptor, a death receptor ligand or a combination thereof. This method can be used to induce cell death in any desired cell and has particular usefulness in the treatment of cancer where cells escape natural apoptotic signals.

[00371] Apoptosis can be induced by multiple independent signaling pathways that converge upon a final effector mechanism consisting of multiple interactions between several "death receptors" and their ligands, which belong to the tumor necrosis factor (TNF) receptor/ligand superfamily. The best-characterized death receptors are CD95 ("Fas"), TNFR1 (p55), death receptor 3 (DR3 or Apo3/TRAMO), DR4 and DR5 (apo2-TRAIL-R2). The final effector mechanism of apoptosis is the activation of a series of proteinases designated as caspases. The activation of these caspases results in the cleavage of a series of vital cellular proteins and cell death. The molecular mechanism of death receptors/ligands-induced apoptosis is well known in the art. For example, Fas/FasL-mediated apoptosis is induced by binding of three FasL molecules which induces trimerization of Fas receptor via C-terminus death domains (DDs), which in turn recruit an adapter protein FADD (Fas-associated protein with death domain) and Caspase-8. The oligomerization of this trimolecular complex, Fas/FAIDD/caspase-8, results in proteolytic cleavage of proenzyme caspase-8 into active caspase-8 that, in turn, initiates the apoptosis process by activating other downstream caspases through proteolysis, including caspase-3. Death ligands in general are apoptotic when formed into trimers or higher order of structures. As monomers, they may serve as antiapoptotic agents by competing with the trimers for binding to the death receptors.

[00372] In one embodiment, the synthetic, modified RNA composition encodes for a death receptor (*e.g.*, Fas, TRAIL, TRAMO, TNFR, TLR etc). Cells made to express a death receptor by transfection of modified RNA become susceptible to death induced by the ligand that activates that receptor. Similarly, cells made to express a death ligand, *e.g.*, on their surface, will induce death of cells with the receptor when the transfected cell contacts the target cell. In another embodiment, the modified RNA composition encodes for a death receptor ligand (*e.g.*, FasL, TNF, etc). In another embodiment, the modified RNA composition encodes a caspase (*e.g.*, caspase 3, caspase 8, caspase 9 etc). Where cancer cells often exhibit a failure to properly differentiate to a non-proliferative or controlled proliferative form, in another embodiment, the synthetic, modified RNA composition encodes for both a death receptor and its appropriate activating ligand. In another embodiment, the synthetic, modified RNA composition encodes for a differentiation factor that when expressed in the

cancer cell, such as a cancer stem cell, will induce the cell to differentiate to a non-pathogenic or non-self-renewing phenotype (*e.g.*, reduced cell growth rate, reduced cell division etc) or to induce the cell to enter a dormant cell phase (*e.g.*, G_0 resting phase).

[00373] One of skill in the art will appreciate that the use of apoptosis-inducing techniques will require that the synthetic, modified RNAs are appropriately targeted to *e.g.*, tumor cells to prevent unwanted wide-spread cell death. Thus, one can use a delivery mechanism (*e.g.*, attached ligand or antibody, targeted liposome etc) that recognizes a cancer antigen such that the modified RNAs are expressed only in cancer cells.

Cellular Therapies and Cellular Administration

[00374] The compositions and methods comprising synthetic, modified RNAs are particularly useful for generating cells, such as differentiated cells, for use in patients in need of cellular therapies or regenerative medicine applications. Accordingly, various embodiments of the methods and compositions described herein involve administration of an effective amount of a cell or a population of cells, generated using any of the compositions or methods comprising synthetic, modified RNAs described herein, to an individual or subject in need of a cellular therapy. The cell or population of cells being administered can be an autologous population, or be derived from one or more heterologous sources. The cell can be, for example, a stem cell, such as a lineage-restricted progenitor cell, multipotent cell, or an oligopotent cell, or a fully or partially differentiated progeny of a stem cell. In some embodiments, the stem cell can be generated through the introduction of synthetic, modified RNAs encoding differentiation factor(s) as described herein. In addition, the population of cells administered can be of a lineage selected from one of an ecotodermal lineage, a mesodermal lineage, or an endodermal lineage. The cell can also be a cell modified to express a targeting moiety or a mediator of targeted cell death, using synthetic, modified RNAs as described herein. Further, such differentiated cells can be administered in a manner that permits them to graft to the intended tissue site and reconstitute or regenerate the functionally deficient area. In some such embodiments, differentiated cells can be introduced to a scaffold or other structure to generate, for example, a tissue *ex vivo*, that can then be introduced to a patient. For example, islet precursor cells or their derivatives can be generated to restore islet function in a patient having any condition relating to inadequate production of a pancreatic endocrine (insulin, glucagon, or somatostatin), or the inability to properly regulate secretion, *e.g.*, Type I (insulin-dependent) diabetes mellitus.

[00375] A variety of means for administering cells to subjects are known to those of skill in the art. Such methods can include systemic injection, for example i.v. injection, or implantation of cells into a target site in a subject. Cells may be inserted into a delivery device which facilitates introduction by injection or implantation into the subject. Such delivery devices can include tubes, *e.g.*, catheters, for injecting cells and fluids into the body of a recipient subject. In one preferred embodiment, the tubes additionally have a needle, *e.g.*, through which the cells can be introduced into

the subject at a desired location. The cells can be prepared for delivery in a variety of different forms. For example, the cells can be suspended in a solution or gel or embedded in a support matrix when contained in such a delivery device. Cells can be mixed with a pharmaceutically acceptable carrier or diluent in which the cells remain viable.

[00376] Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. The solution is preferably sterile and fluid. Preferably, prior to the introduction of cells as described herein, the solution is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi through the use of, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like.

[00377] It is preferred that the mode of cell administration is relatively non-invasive, for example by intravenous injection, pulmonary delivery through inhalation, topical, or intranasal administration. However, the route of cell administration will depend on the tissue to be treated and may include implantation. Methods for cell delivery are known to those of skill in the art and can be extrapolated by one skilled in the art of medicine for use with the methods and compositions described herein.

[00378] Direct injection techniques for cell administration can also be used to stimulate transmigration of cells through the entire vasculature, or to the vasculature of a particular organ, such as for example liver, or kidney or any other organ. This includes non-specific targeting of the vasculature. One can target any organ by selecting a specific injection site, *e.g.*, a liver portal vein. Alternatively, the injection can be performed systemically into any vein in the body. This method is useful for enhancing stem cell numbers in aging patients. In addition, the cells can function to populate vacant stem cell niches or create new stem cells to replenish the organ, thus improving organ function. For example, cells may take up pericyte locations within the vasculature. In another example, neural stem cells or precursor cells generated using the compositions and methods comprising synthetic, modified RNAs are transplanted directly into parenchymal or intrathecal sites of the central nervous system, according to the disease being treated, such as for example, a spinal cord injury. Grafts can be done using single cell suspension or small aggregates at a density of 25,000-500,000 cells per mL (U.S. Pat. No. 5,968,829). A successful transplant can show, for example, transplant-derived cells present in the lesion 2-5 weeks later, differentiated into astrocytes, oligodendrocytes, and/or neurons, and migrating along the cord from the lesioned end.

[00379] If so desired, a mammal or subject can be pre-treated with an agent, for example an agent is administered to enhance cell targeting to a tissue (*e.g.*, a homing factor) and can be placed at that site to encourage cells to target the desired tissue. For example, direct injection of homing factors into a tissue can be performed prior to systemic delivery of ligand-targeted cells.

Scaffolds and Tissue Engineering

[00380] It is further contemplated that, in some embodiments of these aspects, cells generated by differentiation or transdifferentiation using the synthetic, modified RNAs described herein, can not only be administered as cells in suspension, but also as cells populating a matrix, scaffold, or other support to create an artificial tissue, for use in cellular therapies in regenerative medicine and tissue engineering.

[00381] Tissue engineering refers to the use of a combination of cells, engineering and materials methods, and suitable biochemical and physio-chemical factors for the *de novo* generation of tissue or tissue structures. Such engineered tissue or tissue structures are useful for therapeutic purposes to improve or replace biological functions. As used herein, "engineered tissue" encompasses a broad range of applications, including, but not limited to ,utility in the repair or replace portions of, or whole tissues (*e.g.*, heart, cardiac tissue, ventricular myocardium, and other tissues such as bone, cartilage, pancreas, liver, kidney, blood vessels, bladder, *etc.*), or in assays for identifying agents which modify the function of parts of, or entire organs without the need to obtain such organs from a subject.

[00382] In some embodiments, a "support" *i.e.*, any suitable carrier material to which cells generated using the methods and compositions comprising synthetic, modified RNAs described herein are able to attach themselves or adhere, is used in order to form a corresponding cell composite, *e.g.* an artificial tissue. In some embodiments, a matrix or carrier material, respectively, is present already in a three-dimensional form desired for later application. For example, bovine pericardial tissue can be used as matrix which is crosslinked with collagen, decellularized and photofixed.

[00383] In some such embodiments, a scaffold, which can also be referred to as a "biocompatible substrate," can be used as a material that is suitable for implantation into a subject onto which a cell population can be deposited. A biocompatible substrate does not cause toxic or injurious effects once implanted in the subject. In one embodiment, the biocompatible substrate is a polymer with a surface that can be shaped into a desired structure that requires repairing or replacing. The polymer can also be shaped into a part of a structure that requires repairing or replacing. The biocompatible substrate provides the supportive framework that allows cells to attach to it, and grow on it. Cultured populations of cells can then be grown on the biocompatible substrate, which provides the appropriate interstitial distances required for cell-cell interaction.

[00384] A structure or scaffold can be used to aid in further controlling and directing a cell or population of cells undergoing differentiation or transdifferentiation using the compositions and methods described herein. A structure or scaffold, such as a biopolymer structure, can be designed to provide environmental cues to control and direct the differentiation of cells to a functionally active engineered tissue, *e.g.*, multipotent cells undergoing differentiation, using the synthetic, modified RNAs described herein, into ventricular cardiomyocytes to generate a functional, contracting tissue myocardium structure. By "functionally active," it is meant that the cell attached to the scaffold comprises at least one function of that cell type in its native environment. A structure or scaffold can

be engineered from a nanometer to micrometer to millimeter to macroscopic length, and can further comprise or be based on factors such as, but not limited to, material mechanical properties, material solubility, spatial patterning of bioactive compounds, spatial patterning of topological features, soluble bioactive compounds, mechanical perturbation (cyclical or static strain, stress, shear, etc...), electrical stimulation, and thermal perturbation.

[00385] The construction of an engineered tissue can be carried out by first assembling the scaffolds, and then seeding with a cell type that has undergone differentiation or partial differentiation using the synthetic, modified RNA compositions and methods described herein. Alternatively, an engineered tissue can be made by seeding a matrix or other scaffold component cell with cells, such as iPS cells or human ES cells, and applying or introducing a desired synthetic, modified RNA composition directly to the scaffold comprising the cells. A scaffold can be in any desired geometric conformation, for example, a flat sheet, a spiral, a cone, a v-like structure and the like. A scaffold can be shaped into, *e.g.*, a heart valve, vessel (tubular), planar construct or any other suitable shape. Such scaffold constructs are known in the art (see, *e.g.*, WO02/035992, U.S. Pat. Nos. 6,479,064, 6,461,628). In some embodiments, after culturing the cells on the scaffold, the scaffold is removed (*e.g.*, bioabsorbed or physically removed), and the layers of differentiation or transdifferentiated cells maintain substantially the same conformation as the scaffold, such that, for example, if the scaffold was spiral shaped, the cells form a 3D-engineered tissue that is spiral shaped. In addition, it is contemplated that different synthetic, modified RNA compositions can be contacted with or applied to a scaffold comprising cells in order to allow the growth and differentiation of a plurality of different, differentiated cells types to form a desired engineered tissue. For example, for construction of muscle tissue with blood vessels, a scaffold can be seeded with different population of cells which make up blood vessels, neural tissue, cartilage, tendons, ligaments and the like.

[00386] Biopolymer structures can be generated by providing a transitional polymer on a substrate; depositing a biopolymer on the transitional polymer; shaping the biopolymer into a structure having a selected pattern on the transitional polymer (poly(N- Isopropylacrylamide); and releasing the biopolymer from the transitional polymer with the biopolymer' s structure and integrity intact. A biopolymer can be selected from an extracellular matrix (ECM) protein, growth factor, lipid, fatty acid, steroid, sugar and other biologically active carbohydrates, a biologically derived homopolymer, nucleic acids, hormone, enzyme, pharmaceutical composition, cell surface ligand and receptor, cytoskeletal filament, motor protein, silks, polyprotein (*e.g.*, poly(lysine)) or any combination thereof. The biopolymers used in the generation of the scaffolds for the embodiments directed to tissue engineering described herein include, but are not limited to, a) extracellular matrix proteins to direct cell adhesion and function (*e.g.*, collagen, fibronectin, laminin, etc.); (b) growth factors to direct cell function specific to cell type (*e.g.*, nerve growth factor, bone morphogenic proteins, vascular endothelial growth factor, etc.); (c) lipids, fatty acids and steroids (*e.g.*, glycerides,

non-glycerides, saturated and unsaturated fatty acids, cholesterol, corticosteroids, sex steroids, etc.);(d) sugars and other biologically active carbohydrates (*e.g.*, monosaccharides, oligosaccharides, sucrose, glucose, glycogen, etc.); (e) combinations of carbohydrates, lipids and/or proteins, such as proteoglycans (protein cores with attached side chains of chondroitin sulfate, dermatan sulfate, heparin, heparan sulfate, and/or keratan sulfate); glycoproteins [*e.g.*, selectins, immunoglobulins, hormones such as human chorionic gonadotropin, Alpha- fetoprotein and Erythropoietin (EPO), etc.]; proteolipids (*e.g.*, N-myristoylated, palmitoylated and prenylated proteins); and glycolipids (*e.g.*, glycoglycerolipids, glycosphingolipids, glycophosphatidylinositols, etc.); (f) biologically derived homopolymers, such as polylactic and polyglycolic acids and poly-L-lysine; (g) nucleic acids (*e.g.*, DNA, RNA, etc.); (h) hormones (*e.g.*, anabolic steroids, sex hormones, insulin, angiotensin, etc.); (i) enzymes (types: oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases; examples: trypsin, collegenases, matrix metallproteinases, etc.); (j) pharmaceuticals (*e.g.*, beta blockers, vasodilators, vasoconstrictors, pain relievers, gene therapy, viral vectors, anti-inflammatories, etc.); (k) cell surface ligands and receptors (*e.g.*, integrins, selectins, cadherins, etc.); (1) cytoskeletal filaments and/or motor proteins (*e.g.*, intermediate filaments, microtubules, actin filaments, dynein, kinesin, myosin, etc.), or any combination thereof. For example, a biopolymer can be selected from the group consisting of fibronectin, vitronectin, laminin, collagen, fibrinogen, silk or silk fibroin.

[00387] Following or during construction of a biopolymer scaffold, cells can be integrated into or onto the scaffold. In some embodiments, the cells to be differentiated are human ES-derived cells or iPS-derived cells, and the methods further comprise growing the cells in the scaffold where the structure, composition, ECM type, growth factors and/or other cell types can assist in differentiation of the cells into the desired differentiated cell type. In some embodiments, such engineered tissue can be further used in drug screening applications. For example, an engineered myocardium tissue composition can be useful as a tool to identify agents which modify the function of cardiac muscle (*e.g.*, to identify cardiotoxic agents).

[00388] Other exemplary materials suitable for polymer scaffold fabrication include, but are not limited to, polylactic acid (PLA), poly-L-lactic acid (PLLA), poly-D-lactic acid (PDLA), polyglycolide, polyglycolic acid (PGA), polylactide-co-glycolide (PLGA), polydioxanone, polygluconate, polylactic acid-polyethylene oxide copolymers, modified cellulose, collagen, polyhydroxybutyrate, polyhydroxpriopionic acid, polyphosphoester, poly(alpha-hydroxy acid), polycaprolactone, polycarbonates, polyamides, polyanhydrides, polyamino acids, polyorthoesters, polyacetals, polycyanoacrylates, degradable urethanes, aliphatic polyester polyacrylates, polymethacrylate, acyl substituted cellulose acetates, non-degradable polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl flouride, polyvinyl imidazole, chlorosulphonated polyolifins, polyethylene oxide, polyvinyl alcohol, Teflon™, nylon silicon, and shape memory materials, such as poly(styrene-block-butadiene), polynorbornene, hydrogels, metallic alloys, and oligo(ε-

caprolactone)diol as switching segment/oligo(p-dioxyanone)diol as physical crosslink. Other suitable polymers can be obtained by reference to The Polymer Handbook, 3rd edition (Wiley, N.Y., 1989).

[00389] In some embodiments, additional bioactive substances can be added to a biopolymer scaffold comprising cells being differentiated using the synthetic, modified RNA compositions described herein, such as, but not limited to, demineralized bone powder as described in U.S. Pat. No. 5,073,373; collagen, insoluble collagen derivatives, etc., and soluble solids and/or liquids dissolved therein; antiviricides, particularly those effective against HIV and hepatitis; antimicrobials and/or antibiotics such as erythromycin, bacitracin, neomycin, penicillin, polymycin B, tetracyclines, biomycin, chloromycetin, and streptomycins, cefazolin, ampicillin, azactam, tobramycin, clindamycin and gentamycin, etc.; biocidal/biostatic sugars such as dextran, glucose, etc.; amino acids; peptides; vitamins; inorganic elements; co-factors for protein synthesis; hormones; endocrine tissue or tissue fragments; synthesizers; enzymes such as alkaline phosphatase, collagenase, peptidases, oxidases, etc.; polymer cell scaffolds with parenchymal cells; angiogenic agents and polymeric carriers containing such agents; collagen lattices; antigenic agents; cytoskeletal agents; cartilage fragments; living cells such as chondrocytes, bone marrow cells, mesenchymal stem cells; natural extracts; genetically engineered living cells or otherwise modified living cells; expanded or cultured cells; DNA delivered by plasmid, viral vectors or other means; tissue transplants; demineralized bone powder; autogenous tissues such as blood, serum, soft tissue, bone marrow, etc.; bioadhesives; bone morphogenic proteins (BMPs); osteoinductive factor (IFO); fibronectin (FN); endothelial cell growth factor (ECGF); vascular endothelial growth factor (VEGF); cementum attachment extracts (CAE); ketanserin; human growth hormone (HGH); animal growth hormones; epidermal growth factor (EGF); interleukins, *e.g.*, interleukin-1 (IL-1), interleukin-2 (IL-2); human alpha thrombin; transforming growth factor (TGF-beta); insulin-like growth factors (IGF-1, IGF-2); platelet derived growth factors (PDGF); fibroblast growth factors (FGF, BFGF, etc.); periodontal ligament chemotactic factor (PDLGF); enamel matrix proteins; growth and differentiation factors (GDF); hedgehog family of proteins; protein receptor molecules; small peptides derived from growth factors above; bone promoters; cytokines; somatotropin; bone digestors; antitumor agents; cellular attractants and attachment agents; immuno-suppressants; permeation enhancers, *e.g.*, fatty acid esters such as laureate, myristate and stearate monoesters of polyethylene glycol, enamine derivatives, alpha-keto aldehydes, etc.; and nucleic acids. The amounts of such optionally added bioactive substances can vary widely with optimum levels being readily determined in a specific case by routine experimentation.

Diseases Treatable by Cell Transplantation

[00390] A wide range of diseases are recognized as being treatable with cellular therapies. Accordingly, also provided herein are compositions and methods comprising synthetic, modified RNAs for generating cells for use in cellular therapies, such as stem cell therapies. As non-limiting

examples, these include diseases marked by a failure of naturally occurring stem cells, such as aplastic anemia, Fanconi anemia, and paroxysmal nocturnal hemoglobinuria (PNH). Others include, for example: acute leukemias, including acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), acute biphenotypic leukemia and acute undifferentiated leukemia; chronic leukemias, including chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), juvenile chronic myelogenous leukemia (JCML) and juvenile myelomonocytic leukemia (JMML); myeloproliferative disorders, including acute myelofibrosis, angiogenic myeloid metaplasia (myelofibrosis), polycythemia vera and essential thrombocythemia; lysosomal storage diseases, including mucopolysaccharidoses (MPS), Hurler's syndrome (MPS-IH), Scheie syndrome (MPS-IS), Hunter's syndrome (MPS-II), Sanfilippo syndrome (MPS-III), Morquio syndrome (MPS-IV), Maroteaux-Lamy Syndrome (MPS-VI), Sly syndrome, beta-glucuronidase deficiency (MPS-VII), adrenoleukodystrophy, mucolipidosis II (I-cell Disease), Krabbe disease, Gaucher's disease, Niemann-Pick disease, Wolman disease and metachromatic leukodystrophy; histiocytic disorders, including familial erythrophagocytic lymphohistiocytosis, histiocytosis-X and hemophagocytosis; phagocyte disorders, including Chediak-Higashi syndrome, chronic granulomatous disease, neutrophil actin deficiency and reticular dysgenesis; inherited platelet abnormalities, including amegakaryocytosis/congenital thrombocytopenia; plasma cell disorders, including multiple myeloma, plasma cell leukemia, and Waldenstrom's macroglobulinemia. Other malignancies treatable with stem cell therapies include but are not limited to breast cancer, Ewing sarcoma, neuroblastoma and renal cell carcinoma, among others. Also treatable with stem cell therapy are: lung disorders, including COPD and bronchial asthma; congenital immune disorders, including ataxia-telangiectasia, Kostmann syndrome, leukocyte adhesion deficiency, DiGeorge syndrome, bare lymphocyte syndrome, Omenn's syndrome, severe combined immunodeficiency (SCID), SCID with adenosine deaminase deficiency, absence of T & B cells SCID, absence of T cells, normal B cell SCID, common variable immunodeficiency and X-linked lymphoproliferative disorder; other inherited disorders, including Lesch-Nyhan syndrome, cartilage-hair hypoplasia, Glanzmann thrombasthenia, and osteopetrosis; neurological conditions, including acute and chronic stroke, traumatic brain injury, cerebral palsy, multiple sclerosis, amyotrophic lateral sclerosis and epilepsy; cardiac conditions, including atherosclerosis, congestive heart failure and myocardial infarction; metabolic disorders, including diabetes; and ocular disorders including macular degeneration and optic atrophy. Such diseases or disorders can be treated either by administration of stem cells themselves, permitting *in vivo* differentiation to the desired cell type with or without the administration of agents to promote the desired differentiation, or by administering stem cells differentiated to the desired cell type *in vitro*. Efficacy of treatment is determined by a statistically significant change in one or more indicia of the targeted disease or disorder.

Dosage and Administration

[00391] Dosage and administration will vary with the condition to be treated and the therapeutic approach taken in a given instance.

[00392] Depending on the disease or disorder being treated and on the approach being taken, cells over a range of, for example, 2-5 x 10^5, or more, *e.g.*, 1 x 10^6, 1 x 10^7, 1 x 10^8, 5 x 10^8, 1 x 10^9, 5 x 10^9, 1 x 10^{10}, 5 x 10^{10} or more can be administered. Where differentiated cells are to be administered, the dose will most often be higher than where stem cells are administered, because differentiated cells will have reduced or limited capacity for self-renewal compared to stem cells. Repeat administration of differentiated cells may be necessary if the cells are not capable of self-renewal.

[00393] It is contemplated that cells generated by differentiation or transdifferentiation can be administered as cells in suspension, or as cells populating a matrix, scaffold, or other support to create an artificial tissue. To this end, resorbable matrices and scaffolds are known in the art, as are approaches for populating them with cells, as has been described herein. As but one example, matrices fabricated out of silk proteins are well suited as supports for cells, and are known to be well tolerated for implantation. Cells as described herein can be seeded on such matrices either alone or in combination with other cells, including autologous cells from the intended recipient, to provide the necessary environment for growth and maintenance of the cells in the desired differentiated (or non-differentiated) state. It is also contemplated that the cells generated by differentiation or transdifferentiation can be administered to a subject in need thereof, in an encapsulated form, according to known encapsulation technologies, including microencapsulation (see, *e.g.*, U.S. Pat. Nos. 4,352,883; 4,353,888; and 5,084,350). Where the differentiated or transdifferentiated cells are encapsulated, in some embodiments the cells are encapsulated by macroencapsulation, as described in U.S. Pat. Nos. 5,284,761; 5,158,881; 4,976,859; 4,968,733; 5,800,828 and published PCT patent application WO 95/05452. In such embodiments, cells on the order of 1 x 10^6, 1 x 10^7, 1 x 10^8, 5 x 10^8, 1 x 10^9, 5 x 10^9, 1 x 10^{10}, 5 x 10^{10} or more can be administered alone or on a matrix or support.

[00394] In other embodiments, cells can be suspended in a gel for administration to keep them relatively localized.

[00395] The success of treatment can be evaluated by the ordinarily skilled clinician by monitoring one or more symptoms or markers of the disease or disorder being treated by administration of the cells. Effective treatment includes any statistically significant improvement in one or more indicia of the disease or disorder. Where appropriate, a clinically accepted grade or scaling system for the given disease or disorder can be applied, with an improvement in the scale or grade being indicative of effective treatment.

[00396] In those aspects and embodiments where synthetic, modified RNAs are to be administered directly, instead of cells treated with or resulting from treatment with synthetic, modified RNA, the dosages will also vary depending upon the approach taken, the mode of delivery and the

disease to be treated. For example, systemic administration without a targeting approach will generally require greater amounts of synthetic, modified RNA than either local administration or administration that employs a targeting or homing approach. Depending upon the targeted cell or tissue and the mode of delivery, effective dosages of synthetic, modified RNA can include, for example, 1 ng/kg of body weight up to a gram or more per kg of body weight and any amount in between. Preferred amounts can be, for example, in the range of 5 µg/kg body weight to 30 µg/kg of body weight or any amount in between. Dosages in such ranges can be administered once, twice, three times, four times or more per day, or every two days, every three days, every four days, once a week, twice a month, once a month or less frequently over a duration of days, weeks or months, depending on the condition being treated - where the therapeutic approach treats or ameliorates but does not permanently cure the disease or disorder, *e.g.*, where the synthetic, modified RNA effects treatment of a metabolic disorder by expression of a protein that is deficient in the subject, administration of modified RNA can be repeated over time as needed. Where, instead, the synthetic, modified RNA leads to the establishment of a cell compartment that maintains itself and treats the disease or disorder, readministration may become unnecessary. Sustained release formulations of synthetic, modified RNA compositions are specifically contemplated herein. Continuous, relatively low doses are contemplated after an initial higher therapeutic dose.

[00397] A pharmaceutical composition that includes at least one synthetic, modified RNA described herein can be delivered to or administered to a subject by a variety of routes depending upon whether local or systemic treatment is desired and upon the area to be treated. Exemplary routes include parenteral, intrathecal, parenchymal, intravenous, nasal, oral, and ocular delivery routes. Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal or intramuscular injection, or intrathecal or intraventricular administration. A synthetic, modified RNA can be incorporated into pharmaceutical compositions suitable for administration. For example, compositions can include one or more synthetic, modified RNAs and a pharmaceutically acceptable carrier. Supplementary active compounds can also be incorporated into the compositions. Compositions for intrathecal or intraventricular administration of synthetic, modified RNAs can include sterile aqueous solutions that can also contain buffers, diluents and other suitable additives.

[00398] In some embodiments, the effective dose of a synthetic, modified RNA can be administered in a single dose or in two or more doses, as desired or considered appropriate under the specific circumstances. If desired to facilitate repeated or frequent infusions, a non-implantable delivery device, e.g., needle, syringe, pen device, or implantatable delivery device, e.g., a pump, semi-permanent stent (e.g., intravenous, intraperitoneal, intracisternal or intracapsular), or reservoir can be advisable. In some such embodiments, the delivery device can include a mechanism to dispense a unit dose of the pharmaceutical composition comprising a synthetic, modified RNA. In some embodiments, the device releases the pharmaceutical composition comprising a synthetic, modified RNAcontinuously, e.g., by diffusion. In some embodiments, the device can include a sensor that

monitors a parameter within a subject. For example, the device can include pump, e.g., and, optionally, associated electronics. Exemplary devices include stents, catheters, pumps, artificial organs or organ components (e.g., artificial heart, a heart valve, etc.), and sutures.

[00399] As used herein, "topical delivery" can refer to the direct application of a synthetic, modified RNA to any surface of the body, including the eye, a mucous membrane, surfaces of a body cavity, or to any internal surface. Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, sprays, and liquids. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Topical administration can also be used as a means to selectively deliver the synthetic, modified RNA to the epidermis or dermis of a subject, or to specific strata thereof, or to an underlying tissue.

[00400] Formulations for parenteral administration can include sterile aqueous solutions which can also contain buffers, diluents and other suitable additives. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir. For intravenous use, the total concentration of solutes should be controlled to render the preparation isotonic.

[00401] A synthetic, modified RNA can be administered to a subject by pulmonary delivery. Pulmonary delivery compositions can be delivered by inhalation by the patient of a dispersion so that the composition comprising a synthetic, modified RNA, within the dispersion can reach the lung where it can be readily absorbed through the alveolar region directly into the lung cells to directly transfect the lung cells, and/or enter the blood circulation. Direct transfection by inhalation will allow expression of a desired protein, for example CFTR, by the transfected lung cells. Accordingly, pulmonary delivery can be effective both for systemic delivery and for localized delivery to treat diseases of the lungs. Pulmonary delivery can be achieved by different approaches, including the use of nebulized, aerosolized, micellular and dry powder-based formulations of the compositions comprising synthetic, modified RNAs described herein. Delivery can be achieved with liquid nebulizers, aerosol-based inhalers, and dry powder dispersion devices. Metered-dose devices are preferred. One of the benefits of using an atomizer or inhaler is that the potential for contamination is minimized because the devices are self contained. Dry powder dispersion devices, for example, deliver drugs that can be readily formulated as dry powders. A synthetic, modified RNA composition can be stably stored as lyophilized or spray-dried powders by itself or in combination with suitable powder carriers. The delivery of a composition comprising a synthetic, modified RNA for inhalation can be mediated by a dosing timing element which can include a timer, a dose counter, time measuring device, or a time indicator which when incorporated into the device enables dose tracking, compliance monitoring, and/or dose triggering to a patient during administration of the aerosol medicament.

[00402] A synthetic, modified RNA can be modified such that it is capable of traversing the blood brain barrier. For example, the synthetic, modified RNA can be conjugated to a molecule that enables the agent to traverse the barrier. Such conjugated synthetic, modified RNA can be

administered by any desired method, such as by intraventricular or intramuscular injection, or by pulmonary delivery, for example.

[00403] A composition comprising a synthetic, modified RNA described herein can also be delivered through the use of implanted, indwelling catheters that provide a means for injecting small volumes of fluid containing the synthetic, modified RNAs described herein directly into local tissues. The proximal end of these catheters can be connected to an implanted, access port surgically affixed to the patient's body, or to an implanted drug pump located in, for example, the patient's torso.

[00404] Alternatively, implantable delivery devices, such as an implantable pump can be employed. Examples of the delivery devices for use with the compositions comprising a synthetic, modified RNA described herein include the Model 8506 investigational device (by Medtronic, Inc. of Minneapolis, Minn.), which can be implanted subcutaneously in the body or on the cranium, and provides an access port through which therapeutic agents can be delivered. In addition to the aforementioned device, the delivery of the compositions comprising a synthetic, modified RNA described herein can be accomplished with a wide variety of devices, including but not limited to U.S. Pat. Nos. 5,735,814, 5,814,014, and 6,042,579. Using the teachings described herein, those of skill in the art will recognize that these and other devices and systems can be suitable for delivery of compositions comprising the synthetic, modified RNAs described herein.

[00405] In some such embodiments, the delivery system further comprises implanting a pump outside the body, the pump coupled to a proximal end of the catheter, and operating the pump to deliver the predetermined dosage of a composition comprising a synthetic, modified RNA described herein through the discharge portion of the catheter. A further embodiment comprises periodically refreshing a supply of the composition comprising a synthetic, modified RNA to the pump outside said body.

[00406] A synthetic, modified RNA can be administered ocularly, such as to treat retinal disorders, e.g., a retinopathy. For example, the pharmaceutical compositions can be applied to the surface of the eye or nearby tissue, e.g., the inside of the eyelid. They can be applied topically, e.g., by spraying, in drops, as an eyewash, or an ointment. Ointments or droppable liquids can be delivered by ocular delivery systems known in the art, such as applicators or eye droppers. Such compositions can include mucomimetics such as hyaluronic acid, chondroitin sulfate, hydroxypropyl methylcellulose or poly(vinyl alcohol), preservatives such as sorbic acid, EDTA or benzylchronium chloride, and the usual quantities of diluents and/or carriers. The pharmaceutical composition can also be administered to the interior of the eye, and can be introduced by a needle or other delivery device which can introduce it to a selected area or structure. The composition containing the synthetic, modified RNA can also be applied via an ocular patch.

[00407] A synthetic, modified RNA can be administered by an oral or nasal delivery. For example, drugs administered through these membranes have a rapid onset of action, provide

therapeutic plasma levels, avoid first pass effect of hepatic metabolism, and avoid exposure of the drug to the hostile gastrointestinal (GI) environment. Additional advantages include easy access to the membrane sites so that the drug can be applied, localized and removed easily.

[00408] Administration of a composition comprising a synthetic, modified RNA can be provided by the subject or by another person, e.g., a another caregiver. A caregiver can be any entity involved with providing care to the human: for example, a hospital, hospice, doctor's office, outpatient clinic; a healthcare worker such as a doctor, nurse, or other practitioner; or a spouse or guardian, such as a parent. The medication can be provided in measured doses or in a dispenser which delivers a metered dose.

[00409] Where cells expressing proteins encoded by synthetic, modified RNA as described herein are administered to treat a malignancy or disease or disorder, the dose of cells administered will also vary with the therapeutic approach. For example, where the cell expresses a death ligand targeting the tumor cell, the dosage of cells administered will vary with the mode of their administration, *e.g.*, local or systemic (smaller doses are required for local), and with the size of the tumor being treated - generally more cells or more frequent administration is warranted for larger tumors versus smaller ones. The amount of cells administered will also vary with the level of expression of the polypeptide or polypeptides encoded by the modified RNA - this is equally true of the administration of cells expressing proteins encoded by modified RNA for any purpose described herein. An important advantage of the methods described herein is that where, for example, more than one factor or polypeptide is expressed from a modified RNA introduced to a cell, the relative dosage of the expressed proteins can be tuned in a straightforward manner by adjusting the relative amounts of the modified RNAs introduced to the cell or subject. This is in contrast to the difficulty of tuning the expression of even a single gene product in a cell transduced with a viral or even a plasmid vector.

[00410] Therapeutic compositions containing at least one synthetic, modified-NA can be conventionally administered in a unit dose. The term "unit dose" when used in reference to a therapeutic composition refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required physiologically acceptable diluent, *i.e.*, carrier, or vehicle.

[00411] The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered and timing depends on the subject to be treated, capacity of the subject's system to utilize the active ingredient, and degree of therapeutic effect desired.

Pharmaceutical Compositions

[00412] The present invention involves therapeutic compositions useful for practicing the therapeutic methods described herein. Therapeutic compositions contain a physiologically tolerable

carrier together with an active compound (synthetic, modified RNA, a cell transfected with a synthetic, modified RNA, or a cell differentiated, de-differentiated or transdifferentiated with a synthetic, modified RNA) as described herein, dissolved or dispersed therein as an active ingredient. In a preferred embodiment, the therapeutic composition is not immunogenic when administered to a mammal or human patient for therapeutic purposes, unless so desired. As used herein, the terms "pharmaceutically acceptable," "physiologically tolerable," and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a mammal without the production of undesirable or unacceptable physiological effects such as toxicity, nausea, dizziness, gastric upset, immune reaction and the like. A pharmaceutically acceptable carrier will not promote the raising of an immune response to an agent with which it is admixed, unless so desired. The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectable either as liquid solutions or suspensions, however, particularly where synthetic, modified RNA itself is administered, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified or presented as a liposome composition. The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient. Physiologically tolerable carriers are well known in the art. Exemplary liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Saline-based carriers are most useful for the administration of cells or cell preparations. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes.

Kits

[00413] Provided herein are kits comprising synthetic, modified RNAs as described herein and kits for preparing such synthetic, modified RNAs.

[00414] Provided herein, in some aspects, are kits for altering the phenotype or the developmental potential of a cell, and comprise (a) a synthetic, modified RNA composition comprising at least one synthetic, modified RNA molecule comprising: (i) a 5' cap, (ii) an open reading frame encoding a polypeptide, and (iii) at least one modified nucleoside, and (b) packaging and instructions therefor.

[00415] In one embodiment of this aspect, the synthetic, modified RNA composition can further comprise a 3' untranslated region (*e.g.*, murine alpha-globin 3' untranslated region) to enhance the stability of the synthetic, modified RNA. In another embodiment of this aspect, the 5' cap is a 5' cap analog such as *e.g.*, a 5' diguanosine cap, tetraphosphate cap analogs having a methylene-bis(phosphonate) moiety (see *e.g.*, Rydzik, AM et al., (2009) Org Biomol Chem 7(22):4763-76), dinucleotide cap analogs having a phosphorothioate modification (see *e.g.*, Kowalska, J. et al., (2008) RNA 14(6):1119-1131), cap analogs having a sulfur substitution for a non-bridging oxygen (see *e.g.*, Grudzien-Nogalska, E. et al., (2007) RNA 13(10): 1745-1755), N7-benzylated dinucleoside tetraphosphate analogs (see *e.g.*, Grudzien, E. et al., (2004) RNA 10(9):1479-1487), or anti-reverse cap analogs (see *e.g.*, Jemielity, J. et al., (2003) RNA 9(9): 1108-1122 and Stepinski, J. et al., (2001) RNA 7(10):1486-1495).

[00416] In other embodiments, the kit can further comprise materials for further reducing the innate immune response of a cell. For example, the kit can further comprise a soluble interferon receptor, such as B18R. The synthetic, modified RNAs provided in such a kit can encode for a polypeptide to express a transcription factor, a targeting moiety, a cell type-specific polypeptide, a cell-surface polypeptide, a differentiation factor, a reprogramming factor or a de-differentiation factor. The synthetic, modified RNA can be provided such that the synthetic, modified RNA is dephosphorylated, lacks a 5' phosphate, comprises a 5' monophosphate, or lacks a 5' triphosphate.

[00417] In some embodiments, the kit can comprise a plurality of different synthetic, modified RNA molecules.

[00418] In some aspects, the kit can be provided to induce reprogramming of a somatic cell to an induced pluripotent stem cell. Such kits include synthetic, modified RNAs encoding Oct4, Klf4, Sox2, or MYC. In some embodiments, the kits further comprise a synthetic, modified RNAs encoding LIN-28. The kit can provide the synthetic, modified RNAs in an admixture or as separate RNA aliquots.

[00419] The kit can further comprise an agent to enhance efficiency of reprogramming (*e.g.*, valproic acid). The kit can further comprise one or more antibodies or primer reagents to detect a cell-type specific marker to identify reprogrammed cells.

[00420] Also provided herein are kits for preparing a synthetic, modified RNA. The kit comprises at least one modified nucleoside, such as 5'-methylcytidine or pseudouridine and an RNA polymerase. The kit can also comprise a 5' cap analog. The kit can also comprise a phosphatase enzyme (*e.g.*, Calf intestinal phosphatase) to remove the 5' triphosphate during the RNA modification procedure. The kit can also comprise one or more templates for the generation of a synthetic, modified- RNA.

[00421] In one aspect, provided herein are kits comprising: (a) a container or vial with at least one synthetic, modified RNA molecule comprising at least two modified nucleosides, and (b) packaging and instructions therefor. Optionally, the kit can comprise one or more control synthetic,

modified RNAs, such as a synthetic, modified RNA encoding green fluorescent protein (GFP) or other marker molecule. In some embodiments of this aspect, the at least two modified nucleosides are selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2'deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In some embodiments of this aspect, the at least two modified nucleosides are 5-methylcytidine (5mC) and pseudouridine.

[00422] In some embodiments of this aspect, the container with at least one synthetic, modified RNA molecule comprising at least two modified nucleosides further comprises a buffer. In some such embodiments, the buffer is RNase-free TE buffer at pH 7.0. In some embodiments of this aspect, the kit further comprises a container with cell culture medium.

[00423] In some embodiments of this aspect, the at least one synthetic, modified RNA encodes a developmental potential altering factor. In some such embodiments, the developmental potential altering factor is a reprogramming factor, a differentiation factor, or a de-differentiation factor.

[00424] In some embodiments of this aspect, the kit further comprises a container or vial comprising IFN inhibitor. In some embodiments of this aspect, the kit further comprises a container or vial valproic acid.

[00425] In some embodiments of this aspect, the synthetic, modified RNA encoding a reprogramming factor in the vial or container has a concentration of 100 ng/µl.

[00426] In some embodiments of this aspect, the reprogramming factor is selected from the group consisting of: OCT4 (SEQ ID NO: 788), SOX1, SOX 2 (SEQ ID NO: 941 or SEQ ID NO: 1501), SOX 3, SOX15, SOX 18, NANOG, KLF1, KLF 2, KLF 4 (SEQ ID NO: 501), KLF 5, NR5A2, c-MYC (SEQ ID NO: 636), l- MYC, n- MYC, REM2, TERT, and LIN28 (SEQ ID NO: 524). In some such embodiments, the kit comprises at least three of the reprogramming factors selected from the group consisting of OCT4, SOX1, SOX 2, SOX 3, SOX15, SOX 18, NANOG, KLF1, KLF 2, KLF 4, KLF 5, NR5A2, c-MYC, l- MYC, n-MYC, REM2, TERT, and LIN28. In some embodiments, the kit does not comprise a synthetic, modified RNA encoding c-MYC.

[00427] In some embodiments of those aspects where the kit is provided to induce reprogramming of a somatic cell to an induced pluripotent stem cell, the kit comprises: a vial comprising a synthetic, modified RNA encoding OCT4 and a buffer; a vial comprising a synthetic, modified RNA encoding SOX2 and a buffer; a vial comprising a synthetic, modified RNA encoding c-MYC and a buffer; and a vial comprising a synthetic, modified RNA encoding KLF4 and a buffer.

In some such embodiments, the concentration of each reprogramming factor in the vial is 100 ng/µl. In some embodiments, the at least two modified nucleosides are pseudouridine and 5-methylcytodine. In some embodiments, OCT4 is provided in the kit in a molar excess of about three times the concentration of KLF4, SOX-2, and c-MYC in the kit. In some such embodiments, the kit further comprises a vial comprising a synthetic, modified RNA molecule encoding LIN28 and a buffer. In some such embodiments, the buffer is RNase-free TE buffer at pH 7.0. In some embodiments, the kit further comprises a synthetic, modified RNA encoding a positive control molecule, such as GFP.

[00428] For example, in one embodiment of those aspects where the kit is provided to induce reprogramming of a somatic cell to an induced pluripotent stem cell, the kit comprises: a vial comprising a synthetic, modified RNA encoding OCT4 and a buffer; a vial comprising a synthetic, modified RNA encoding SOX2 and a buffer; a vial comprising a synthetic, modified RNA encoding c-MYC and a buffer; a vial comprising a synthetic, modified RNA encoding KLF4 and a buffer; a vial comprising a synthetic, modified RNA molecule encoding LIN28 and a buffer; a vial comprising a synthetic, modified RNA encoding a positive control GFP molecule; and packaging and instructions therefor; where the concentration of the synthetic, modified RNAs encoding OCT4, SOX2, c-MYC, KLF-4, LIN28 and GFP in each of the said vials is 100 ng/µl, wherein the buffers in each of said vials is RNase-free TE buffer at pH 7.0; and wherein the synthetic, modified RNAs encoding OCT4, SOX2, c-MYC, KLF-4, LIN28 and GFP all comprise pseudouridine and 5-methylcytidine nucleoside modifications.

[00429] In other embodiments of those aspects where the kit is provided to induce reprogramming of a somatic cell to an induced pluripotent stem cell, the kit comprises: a single container or vial comprising all the synthetic, modified RNAs provided in the kit. In some such embodiments, the kit comprises a single vial or single container comprising: a synthetic, modified RNA encoding OCT4; a synthetic, modified RNA encoding SOX2; a synthetic, modified RNA encoding c-MYC; a synthetic, modified RNA encoding KLF4; and a buffer. In some such embodiments, the buffer is RNase-free TE buffer at pH 7.0. In some such embodiments, the total concentration of reprogramming factors in the vial is 100 ng/µl. In some embodiments, the at least two modified nucleosides are pseudouridine and 5-methylcytodine. In some such embodiments, OCT4 is provided in the vial or container in a molar excess of about three times the concentration of KLF4, SOX-2, and c-MYC in the vial or container. In some such embodiments, the vial or container further comprises a synthetic, modified RNA molecule encoding LIN28. In some such embodiments, the buffer is RNase-free TE buffer at pH 7.0. In some embodiments, the kit further comprises a synthetic, modified RNA encoding a positive control molecule, such as GFP.

[00430] In some embodiments, the kits provided herein comprise at least one synthetic, modified RNA further comprising a 5' cap. In some such embodiments, the 5' cap is a 5' cap analog. In some such embodiments, the 5' cap analog is a 5' diguanosine cap.

[00431] In some embodiments, t the kits provided herein comprise at least one synthetic, modified RNA that does not comprise a 5' triphosphate.

[00432] In some embodiments, the kits provided herein comprise at least one synthetic and modified RNA further comprising a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated regions, or any combination thereof. In some such embodiments, the poly(A) tail, the Kozak sequence, the 3' untranslated region, the 5' untranslated region, or the any combination thereof, comprises one or more modified nucleosides.

[00433] All kits described herein can further comprise a buffer, a cell culture medium, a transfection medium and/or a media supplement. In preffered embodiments, the buffers, cell culture mediums, transfection mediums, and/or media supplements are RNase-free. In some embodiments, the synthetic, modified RNAs provided in the kits can be in a non-solution form of specific quantity or mass, e.g., 20 μg, such as a lyophilized powder form, such that the end-user adds a suitable amount of buffer or medium to bring the synthetic, modified RNAs to a desired concentration, e.g., 100 ng/μl.

[00434] All kits described herein can further comprise devices to facilitate single-adminstration or repeated or frequent infusions of a synthetic, modified RNA, such as a non-implantable delivery device, e.g., needle, syringe, pen device, or an implantatable delivery device, e.g., a pump, semi-permanent stent (e.g., intravenous, intraperitoneal, intracisternal or intracapsular), or reservoir. In some such embodiments, the delivery device can include a mechanism to dispense a unit dose of a composition comprising a synthetic, modified RNA. In some embodiments, the device releases the composition comprising a synthetic, modified RNA continuously, e.g., by diffusion. In some embodiments, the device can include a sensor that monitors a parameter within a subject. For example, the device can include pump, e.g., and, optionally, associated electronics.

Screening Methods

[00435] The ability to safely and efficiently reprogram, differentiate, transdifferentiate cells using the synthetic, modified RNAs compositions and methods thereof described herein, as well as generate engineered tissues using such cells, compositions and methods, has high applicability for use in high-throughput screening technologies of disease model systems and assays for the characterization of candidate agents for identifying novel agents for use in the treatment of human disease. Such screening methods and platforms can be used, for example, to identify novel agents for treating a desired disorder; to identify novel agents involved in reprogramming and differentiation, and/or alteration/maintenance of developmental states; or to identify effects of a candidate agent on one or more parameters of a particular cell type or engineered tissue generated using the compositions and methods described herein. Characterization of candidate agents can include aspects such as compound development, identifying cell-specific toxicity and cell-specific survival, and assessments of compound safety, compound efficacy, and dose–response parameters. For example, an engineered

myocardium tissue can be contacted with a test agent, and the effect, if any, of the test agent on a parameter, such as an electrophysiological parameter, associated with normal or abnormal myocardium function, such as contractibility, including frequency and force of contraction, can be determined, or *e.g.*, whether the agent has a cardiotoxic effect.

[00436] The drug discovery process is time-consuming and costly, in part owing to the high rate of attrition of compounds in clinical trials. Thus, modifications and alternative platforms that could accelerate the advancement of promising drug candidates, or reduce the likelihood of failure, would be extremely valuable. High-throughput screening technologies refer to the platforms and assays used to rapidly test thousands of compounds. For example, reporter systems used in cell lines can be used to assess whether compounds activate particular signaling pathways of interest.

[00437] The compositions and methods using synthetic, modified RNAs for reprogramming, differentiating, and transdifferentiating cells, as well as generating engineered tissues, described herein provide a reliable source of cells that can be generated and expanded in an efficient manner to quantities necessary for drug screening and toxicology studies. Further, because the compositions and methods comprising synthetic, modified RNAs described herein minimize the cellular interferon responses, and do not result in permanent genome modifications, the effects of a candidate agent can be studied with minimal confounding factors. As has been described herein, cells can be differentiated to generate specific cell types (for example, neurons, blood cells, pancreatic islet cells, muscle cells, and cardiomyocytes), and induced pluripotent stem cells can be generated from patients with specific diseases, such as, for example, a patient with cystic fibrosis, as demonstrated herein.

[00438] One particular advantage of cells and engineered tissues generated using the compositions, methods, and kits comprising synthetic, modified RNAs described herein for use in screening platforms, is that from a single and potentially limitless starting source, most of the major cells within the human body that could be affected by a drug or other agent can be produced. Such cells provide a better predictive model of both drug efficacy and toxicity than rodent cell lines or immortalized human cell lines that are currently used in high-throughput screens. While such immortalized cell and animal models have contributed a wealth of information about the complexity of various disease processes, compounds that show a significant benefit in such models can fail to show effectiveness in clinical trials. For example, use of a transgenic mouse that overexpresses mutant superoxide dismutase (SOD), a gene found to be associated with amyotrophic lateral sclerosis, enabled the identification of several compounds that alter disease characteristics, including vitamin E and creatine. However, when these compounds were tested in humans, no clinical improvements were observed (A.D. Ebert and C.N. Svendsen, "Human stem cells and drug screening: opportunities and challenges." 2010 Nature Reviews Drug Discovery 9, p. 1-6). Furthermore, toxic effects of compounds are often missed in cell and animal models due to specific interactions with human biological processes that cannot be recapitulated in these systems.

[00439] Accordingly, in some aspects, the compositions comprising synthetic, modified RNAs, and the methods described herein, can be used for evaluating the effects of novel candidate agents and compounds on specific human cell types that are relevant to drug toxicity effects. In some embodiments, cells can be induced to undergo differentiation to a particular cell type or tissue, using the synthetic, modified RNAs described herein, that the test drug or compound is discovered or known to affect, and then used for performing dose–response toxicity studies. In such embodiments, human stem cells, such as iPS cells, derived from patients can be exposed to appropriate differentiation factors using the compositions and methods comprising synthetic, modified RNAs described herein, and instructed to form the various cell types found in the human body, which could then be useful for assessing multiple cellular parameters and characteristics upon exposure to a candidate agent or compound. For example, the cells could be used to assess the effects of drug candidates on functional cardiomyocytes, or on cardiomyocytes having a specific genetic mutation, because drug development is often stalled by adverse cardiac effects. Thus, measurable disruption of electrophysiological properties by known and novel agents and compounds can be assessed in a clinically relevant, consistent, and renewable cell source. Also, for example, such cells can be used to identify metabolic biomarkers in neural tissues derived from human stem cells after toxin exposure. Such embodiments allow potentially toxic compounds to be eliminated at an early stage of the drug discovery process, allowing efforts to be directed to more promising candidates. As another example, islet cells generated using the methods and compositions comprising synthetic, modified RNAs described herein can be used to screen candidate agents (such as solvents, small molecule drugs, peptides, polynucleotides) or environmental conditions (such as culture conditions or manipulation) that affect the characteristics of islet precursor cells and their various progeny. For example, islet cell clusters or homogeneous β cell preparations can be tested for the effect of candidate agents, such as small molecule drugs, that have the potential to up- or down-regulate insulin synthesis or secretion. The cells are combined with the candidate agent, and then monitored for change in expression or secretion rate of insulin, using, for example, RT-PCR or immunoassay of the culture medium.

[00440] In other aspects, the compositions comprising synthetic, modified RNAs, and the methods thereof described herein, are used in differentiation screens, *i.e.*, for identifying compounds that increase self-renewal or differentiation, promote maturation, or enhance cell survival of cells, such as stem cells, differentiated cells, or cancer cells.

[00441] In other aspects, the compositions comprising the synthetic, modified RNAs, and the methods thereof, described herein, can be used to screen for drugs that may correct an observed disease phenotype. In such aspects, cells can be expanded, differentiated into the desired cell type using synthetic, modified RNAs, and then used to screen for drugs that may correct the observed disease phenotype. A candidate agent or drug can be used to directly contact the surface of a reprogrammed, differentiated, transdifferentiated cell population, or engineered tissue by applying the

candidate agent to a media surrounding the cell or engineered tissue. Alternatively, a candidate agent can be intracellular as a result of introduction of the candidate agent into a cell.

[00442] As used herein, "cellular parameters" refer to quantifiable components of cells or engineered tissues, particularly components that can be accurately measured, most desirably in a high-throughput system. A cellular parameter can be any measurable parameter related to a phenotype, function, or behavior of a cell or engineered tissue. Such cellular parameters include, changes in characteristics and markers of a cell or cell population, including but not limited to changes in viability, cell growth, expression of one or more or a combination of markers, such as cell surface determinants, such as receptors, proteins, including conformational or posttranslational modification thereof, lipids, carbohydrates, organic or inorganic molecules, nucleic acids, *e.g.* mRNA, DNA, global gene expression patterns, etc. Such cellular parameters can be measured using any of a variety of assays known to one of skill in the art. For example, viability and cell growth can be measured by assays such as Trypan blue exclusion, CFSE dilution, and ^{3}II incorporation. Expression of protein or polyeptide markers can be measured, for example, using flow cytometric assays, Western blot techniques, or microscopy methods. Gene expression profiles can be assayed, for example, using microarray methodologies and quantitative or semi-quantitiative real-time PCR assays. A cellular parameter can also refer to a functional parameter, such as a metabolic parameter (e.g., expression or secretion of a hormone, such as insulin or glucagon, or an enzyme, such as carboxypeptidase), an electrophysiological parameter (e.g., contractibility, such as frequency and force of mechanical contraction of a muscle cell; action potentials; conduction, such as conduction velocity), or an immunomodulatory parameter (e.g., expression or secretion of a cytokine or chemokine, such as an interferon, or an interleukin; expression or secretion of an antibody; expression or secretion of a cytotoxin, such as perforin, a granzyme, and granulysin; and phagocytosis).

[00443] The "candidate agent" used in the screening methods described herein can be selected from a group of a chemical, small molecule, chemical entity, nucleic acid sequences, an action; nucleic acid analogues or protein or polypeptide or analogue of fragment thereof. In some embodiments, the nucleic acid is DNA or RNA, and nucleic acid analogues, for example can be PNA, pcPNA and LNA. A nucleic acid may be single or double stranded, and can be selected from a group comprising; nucleic acid encoding a protein of interest, oligonucleotides, PNA, etc. Such nucleic acid sequences include, for example, but not limited to, nucleic acid sequence encoding proteins that act as transcriptional repressors, antisense molecules, ribozymes, small inhibitory nucleic acid sequences, for example but not limited to RNAi, shRNAi, siRNA, micro RNAi (mRNAi), antisense oligonucleotides etc. A protein and/or peptide agent or fragment thereof, can be any protein of interest, for example, but not limited to; mutated proteins; therapeutic proteins; truncated proteins, wherein the protein is normally absent or expressed at lower levels in the cell. Proteins of interest can be selected from a group comprising; mutated proteins, genetically engineered proteins, peptides, synthetic peptides, recombinant proteins, chimeric proteins, antibodies, humanized proteins,

humanized antibodies, chimeric antibodies, modified proteins and fragments thereof. A candidate agent also includes any chemical, entity or moiety, including without limitation synthetic and naturally-occurring non-proteinaceous entities. In certain embodiments, the candidate agent is a small molecule having a chemical moiety. Such chemical moieties can include, for example, unsubstituted or substituted alkyl, aromatic, or heterocyclyl moieties and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, frequently at least two of the functional chemical groups, including macrolides, leptomycins and related natural products or analogues thereof. Candidate agents can be known to have a desired activity and/or property, or can be selected from a library of diverse compounds.

[00444] Also included as candidate agents are pharmacologically active drugs, genetically active molecules, etc. Such candidate agents of interest include, for example, chemotherapeutic agents, hormones or hormone antagonists, growth factors or recombinant growth factors and fragments and variants thereof. Exemplary of pharmaceutical agents suitable for use with the screening methods described herein are those described in, "The Pharmacological Basis of Therapeutics," Goodman and Gilman, McGraw-Hill, New York, N.Y., (1996), Ninth edition, under the sections: Water, Salts and Ions; Drugs Affecting Renal Function and Electrolyte Metabolism; Drugs Affecting Gastrointestinal Function; Chemotherapy of Microbial Diseases; Chemotherapy of Neoplastic Diseases; Drugs Acting on Blood-Forming organs; Hormones and Hormone Antagonists; Vitamins, Dermatology; and Toxicology, all of which are incorporated herein by reference in their entireties. Also included are toxins, and biological and chemical warfare agents, for example see Somani, S. M. (Ed.), "Chemical Warfare Agents," Academic Press, New York, 1992), the contents of which is herein incorporated in its entirety by reference.

[00445] Candidate agents, such as chemical compounds, can be obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds, including biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs. Synthetic chemistry transformations and protecting group methodologies (protection and deprotection) useful in synthesizing the candidate compounds for use in the screening methods described herein are known in the art and include, for example, those such as described in R. Larock (1989) Comprehensive Organic Transformations, VCH Publishers; T. W. Greene and P. G. M. Wuts, Protective Groups in Organic Synthesis, 2nd ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, Fieser and Fieser's Reagents for Organic Synthesis, John Wiley and Sons (1994); and L.

Paquette, ed., Encyclopedia of Reagents for Organic Synthesis, John Wiley and Sons (1995), and subsequent editions thereof.

[00446] Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:6909; Erb et al. (1994) Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann et al. (1994) J. Med. Chem. 37:2678; Cho et al. (1993) Science 261:1303; Carrell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2059; Carell et al (1994) Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop et al. (1994) J. Med. Chem. 37:1233.

[00447] Libraries of candidate agents can be presented in solution (*e.g.*, Houghten (1992), Biotechniques 13:412-421), or on beads (Lam (1991), Nature 354:82-84), chips (Fodor (1993) Nature 364:555-556), bacteria (Ladner, U.S. Pat. No. 5,223,409), spores (Ladner U.S. Pat. No. 5,223,409), plasmids (Cull et al. (1992) Proc Natl Acad Sci USA 89:1865-1869) or on phage (Scott and Smith (1990) Science 249:386-390; Devlin (1990) Science 249:404-406; Cwirla et al. (1990) Proc. Natl. Acad. Sci. 87:6378-6382; Felici (1991) J. Mol. Biol. 222:301-310; Ladner supra).

Polypeptides to be Expressed

[00448] Essentially any polypeptide can be expressed using the synthetic, modified, RNAs described herein. Polypeptides useful with the methods described herein include, but are not limited to, transcription factors, targeting moieties and other cell-surface polypeptides, cell-type specific polypeptides, differentiation factors, death receptors, death receptor ligands, reprogramming factors, and/or de-differentiation factors.

Transcription Factors

[00449] In some embodiments, a synthetic, modified RNA or composition thereof encodes for a transcription factor. As used herein the term "transcription factor" refers to a protein that binds to specific DNA sequences and thereby controls the transfer (or transcription) of genetic information from DNA to mRNA. In one embodiment, the transcription factor encoded by the synthetic, modified RNA is a human transcription factor, such as those described in *e.g.*, Messina DM, et al. (2004) *Genome Res.* 14(10B):2041-2047.

[00450] Some non-limiting examples of human transcription factors (and their mRNA IDs and sequence identifiers) for use in the aspects and embodiments described herein include those listed herein in Table 1 (**SEQ ID NOs: 1-1428 and 1483-1501**).

Table 1: Exemplary Human Transcription Factors

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
AA125825		AA125825	1	Other	
AA634818		AA634818	2	Other	
AATF		NM_012138	3	bZIP	apoptosis antagonizing transcription factor
AB002296	NT_033233:4	AB002296	4	Bromodomain	
AB058701	NT_025741:494	AB058701	5	ZnF-Other	
AB075831	NT_011139:311	AB075831	6	ZnF-C2H2	
ABT1		NM_013375	7	Other	activator of basal transcription 1
ADNP		NM_015339	8	Homeobox	activity-dependent neuroprotector
AEBP2	NT_035211:21	NM_153207	9	ZnF-C2H2	AE(adipocyte enhancer)-binding protein 2
AF020591		NM_014480	10	ZnF-C2H2	zinc finger protein
AF0936808		NM_013242	11	Other	similar to mouse Gir3 or D. melanogaster transcription factor IIB
AF5Q31		NM_014423	12	Structural	ALL 1 fused gene from 5q31
AHR		NM_001621	13	bHLH	aryl hydrocarbon receptor
AHRR	NT_034766:39	NM_020731	14	Co-repressor	aryl hydrocarbon receptor repressor
AI022870		AI022870	15	Other	catalytic subunit of DNA polymerase zeta
AI352508		AI352508	16	Other	Highly similar to DPOZ_HUMAN DNA POLYMERASE ZETA SUBUNIT
AI569906		AI569906	17	ZnF-C2H2	Weakly similar to ZN42_HUMAN ZINC FINGER PROTEIN 42
AIRE		NM_000383	18	ZnF-PHD	autoimmune regulator (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy)
AK024238	NT_023124:29	AK024238	19	Homeobox	
AK056369	NT_034877:1	AK056369	20	ZnF-C2H2	
AK057375	NT_008389:5	AK057375	21	ZnF-C2H2	
AK074366	NT_005825:35	AK074366	22	ZnF-C2H2	
AK074859	NT_011150:41	AK074859	23	ZnF-C2H2	
AK092811	NT_017568:327	AK092811	24	ZnF-C2H2	
AK096221	NT_035560:44	AK096221	25	ZnF-C2H2	
AK096288	NT_007819:700	AK096288	26	ZnF-C2H2	
AK098183	NT_011104:165	AK098183	27	ZnF-C2H2	
AK122874	NT_011568:219	AK122874	28	ZnF-C2H2	
AK126753	NT_011176:418	AK126753	29	ZnF-C2H2	
ANP32A		NM_006305	30	Co-activator	phosphoprotein 32 family, member A
APA1		NM_021188	31	ZnF-C2H2	ortholog of mouse another partner for ARF 1
Apg4B		NM_013325	32	Other	Apg4/Au2 homolog 2 (yeast)
AR		NM_000044	33	NHR	androgen receptor (dihydrotestosterone receptor)

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
ARC		NM_015193	34	Other	activity-regulated cytoskeleton-associated protein
ARIDIA	NT_028053:228	NM_006015	35	Structural	AT rich interactive domain 1A (SWI-like)
ARIH2		NM_006321	36	ZnF-Other	ariedne (drosophila) homolog 2
ARIX		NM_005169	37	Homeobox	aristaless homeobox
ARNT		NM_001668	38	bHLH	aryl hydrocarbon receptor nuclear translocator
ARNT2		NM_014862	39	bHLH	aryl hydrocarbon receptor nuclear translocator 2
ARNTL		NM-001178	40	bHLH	aryl hydrocarbon receptor nuclear translocator-like
ARNTL2	NT_035213:171	NM_020183	41	bHLH	aryl hydrocarbon receptor nuclear translocator-like 2
ARX	NT_025940:10	NM_139058	42	Homeobox	aristaless related homeobox
ASCL1		NM_004316	43	bHLH	achaete-scute complex (Drosophila) homolog-like 1
ASCL2		NM_005170	44	bHLH	achaete-scute complex (Drosophila) homolog-like 2
ASCL3		NM_020646	45	bHLH	achaete-scute complex (Drosophila) homolog-like 3
ASH1		NM-018489.2	46	ZnF-PHD	hypothetical protein ASH1
ASH2L		NM_004674	47	Structural	Ash2 (absent, small, or homeotic, Drosophila, homolog)-like
ATBF1		NM_006885	48	ZnF-C2H2	AT-binding transcription factor 1
ATF1		NM_005171	49	bZIP	activating transcription factor 1
ATF2		NM_001880	50	bZIP	activating transcription factor 2
ATF3		NM_001674	51	bZIP	activating transcription factor 3
ATF4		NM_001675	52	bZIP	Activating transcription factor 4 (tax-responsive enhancer element B67)
ATF5		NM_012068	53	bZIP	activating transcripton factor 5
ATF6		NM_007348	54	bZip	activating transcription factor 6
AW875035		AW875035	55	AnF-C2H2	Moderately similar to YY1, Very very hypothetical protein RMSA-1
AWP1		NM_019006	56	ZnF-AN1	protein associated with PRK1
AY026053	NT_011519:29	AY026053	57	Heat Shock	
BA044953	NT_005825:31	AB079778.1 U26914.1	1497 1498		OSZF isoform; ras-responsive element binding protein (RREB-1)

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
BACH1		NM_001186	58	bZIP	BTB and CNC homology 1, basic leucine zipper transcription factor 1
BACH2		NM_021813	59	bZIP	BTB and CNC homology 1, basic leucine zipper transcription factor 2
BAGE2	NT_029490:10	NM_182482	60	ZnF-PHD	B melanoma antigen family, member 2
BANP		NM_017869	61	Co-activator	BANP homolog, SMAR1 homolog
BAPX1		NM_001189	62	Homeobox	bagpipe homeobox (Drosophila) homolog 1
BARHL1		NM_020064	63	Homeobox	BarH (Drosophila)-like 1
BARHL2		AJ251753	64	Homeobox	BarH (Drosophila)-like 2
BARX1		NM_021570	65	Homeobox	BarH-like homeobox 1
BARX2		NM_003658	66	Homeobox	BarH-like homeobox 2
BATF		NM_006399.3	67	bZIP	basic leucine zipper transcription factor, ATF-like
BAZ1A		NM_013448	68	Bromodomain	bromodomain adjacent to zinc finger domain, 1A
BAZ1B		NM_023005	69	Bromodomain	bromodomain adjacent to zinc finger domain, 1B
BAZ2A		NM_013449	70	Bromodomain	bromodomain adjacent to zinc finger domain, 2A
BAZ2B		NM_013450.2	71	Bromodomain	bromodomain adjacent to zinc finger domain, 2B
BCL11A		NM_018014	72	ZnF-C2H2	B-cell CLL/lymphoma 11A (zinc finger protein)
BCL11B		NM_022898	73	ZnF-C2H2	B-cell CLL/lymphoma 11B (zinc finger protein)
BHLHB3		NM_030762	74	bHLH	basic helix-loop-helix domain containing, class B, 3
BHLHB5		NM_152414	75	bHLH	basic helix-loop-helix domain containing, class B, 5
BIA2	NT_029870:6	NM_015431	76	Co-activator	BIA2 protein
BIZF1		NM_003666	77	bZIP	Basic leucine zipper nuclear factor 1 (JEM-1)
BMI1		NM_005180	78	ZnF-Other	murine leukemia viral (bmii-1) oncogene homolog
BNC		NM_001717	79	Znf-C2H2	basonuclin
BRD1		NM_014577	80	Bromodomain	bromodomain-containing 1
BRD2		NM_005104	81	Bromodomain	bromodomain-containing 2
BRD3		NM_007371	82	Bromodomain	bromodomain-containing 3
BRD4		NM_014299	83	Bromodomain	bromodomain-containing 4
BRD7		NM_013263	84	Bromodomain	bromodomain-containing 7
BRD9	NT_034766:148	NM_023924	85	Bromodomain	bromodomain-containing 9

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
BRDT		NM_001726	86	Bromodomain	Bromodomain, testis-specific
BRF1		NM_001519	87	Other	BRF1 homolog, subunit of RNA polymerase III transcription initiation factor IIIB
BRF2		NM_006887	88	ZnF-C3H	zinc finger protein 36, C3H type-like 2
BRPF1		NM_004634	89	Bromodomain	bromodomain and PHD finger containing, 1
BRPF3		AB033112	90	Bromodomain	bromodomain and PHD finger containing, 3
BS69		NM_006624	91	ZnF-NYND	Adenovirus 5 E1A binding protein
BTAF1		AF038362	92	Other	BTAF1 RNA polymerase II, B-TF11D transcription factor-associated, 170kDa
BTBD1	NT_019601:32	NM_025238	93	ZnF-BTB/POZ	BTB (POZ) domain containing 1
BTBD14A	NT_019501:127	NM_144653	94	ZnF-BTB/POZ	BTB (POZ) domain containing 14A
BTBD14B	NT_031915:27	NM_052876	95	ZnF-BTB/POZ	BTB (POZ) domain containing 14B
BTBD2	NT_011268:135	NM_017797	96	ZnF-BTB/POZ	BTB (POZ) domain containing 2
BTBD3		NM_014962	97	ZnF-BTB/POZ	BTB (POZ) domain containing 3
BTBD4	NT_033241:138	AK023564	98	ZnF-BTB/POZ	BTB (POZ) domain containing 4
BTF3L2		M90355	99	Other	basic transcription factor 3, like 2
BTF3L3		N90356	100	Other	Basic transcription factor 3, like 3
BX538183	NT_011109:1331	BX538183	101	ZnF-C2H2	
BX548737	NT_006802:14	BX648737	102	ZnF-C2H2	
C11orf13		NM_003475	103	Other	chromosome 11 open reading frame 13
C11orf9		NM_013279	104	Other	chromosome 11 open reading frame 9
C14orf101		NM_017799	105	Other	chromosome 14 open reading frame 101
C14orf106		NM_018353	106	Other	chromosome 14 open reading frame 106
C14orf44	NT_010422:242	NM_024731	107	ZnF-BTB/POZ	chromosome 16 open reading frame 44
C1orf2		NM_006589	108	Other	chromosome 10 open reading frame 2
C20orf174		AL713683	109	ZnF-C2H2	chromosome 20 open reading frame174
C21orf18		NM_017438	110	Other	chromosome 21 open reading frame 18
C31P1	NT_034563:155	NM_021633	111	ZnF-BTB/POZ	kelch-like protein C31P1
C5orf7		NM_016604	112	Jumonji	chromosome 5 open reading frame 7
CART1		NM_006982	113	Homeobox	cartilage paired-class

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					homeoprotein 1
CBF2		NM_005760	114	Beta-scaffold-CCAAT	CCAAT-box-binding transcription factor
CBFA2T1		NM_004349	115	ZnF-MYND	core-binding factor, runt domain, alpha subunit 2; translocated to, 1; cyclin D-related
CBFA2T2	NT_028392:284	NM_005093	116	ZnF-MYND	core-binding factor, runt domain, alpha subunit 2; translocated to, 2
CBFA2T3		NM_005187	117	ZnF-MYNC	Core-binding factor, runt domain, alpha subunit 2; translocated to, 3
CBX1		NM_006807	118	Structural	chromobox homolog 1 (Drosophila HP1 beta)
CBX2		X77824	119	Structural	chromobox homolog 2 (Drosophila Pc class))
CBX3		NM_007276	120	Structural	chromobox homolog 3 (Drosophila HP1 gamma)
CBX4		NM_003655	121	Structural	chromobox homolog 4 (Drosophila Pc class)
CBX5		NM_012117	122	Structural	chromobox homolog 5 (Drosophila HP1 alpha)
CBX6		NM_014292	123	Structural	chromobox homolog 6
CBX7		NM_175709	124	Structural	chromobox homolog 7a)
CDX1		NM_001804	125	Homeobox	caudal-type homeobox transcription factor 1
CDX2		NM_001265	126	Homeobox	caudal-type homeobox transcription factor 2
CDX4		NM_005193	127	Homeobox	caudal-type homeobox transcription factor 4
CEBPA		NM_004364	128	bZIP	CCAA T/enhancer binding protein (C/EBP), alpha
CEBPB		NM_005194	129	bZIP	CCAA T/enhancer binding protein (C/EBP), beta
CEBPD		NM_005195	130	bZIP	CCAA T/enhancer binding protein (C/EBP), delta
CEBPE		NM_001805	131	bZIP	CCAA T/enhancer binding protein (C/EBP), epsilon
CEBPG		NM_001806	132	bZIP	CCAA T/enhancer binding protein (C/EBP), gamma
CECR6		Nm_031890	133	Bromodomain	cat eye syndrome chromosome region, candidate 6
CERD4		NM_012074	134	ZnF-PHD	D4, zinc and double PHD fingers, family 3
CEZANNE		NM_020205	135	Co-repressor	cellular zinc finger anti-NF-KappaB Cezanne
CG9879		A1217897		Other	CG9879 (fly) homolog
CGI-149		NM_016079	137	Other	CGI-149 protein
CGI-85		NM_017635	138	Structural	CGI-85 protein
CGI-99		NM_016039	139	Other	CGI-99 protein
CHD1		NM_001270	140	Structural	chromodomain helicase DNA binding protein 1
CHD1L		NM_024568	141	Structural	chromodomain helicase DNA binding protein 1-

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					like
CHD2		NM_001271	142	Structural	chromodomain helicase DNA binding protein 2
CHD3		NM_001272	143	Structural	chromodomain helicase DNA binding protein 3
CHD4		NM_001273	144	Structural	chromodomain helicase DNA binding protein 4
CHD5		NM_015557	145	Structural	chromodomain helicase DNA binding protein 5
CHD6		NM_032221	146	Structural	chromodomain helicase DNA binding protein6
CHES1		NM_005197	147	Forkhead	checkpoint suppressor 1
CHX10		XM_063425	148	Homeobox	ceh-10 homeo domain containing homolog (C. elegans)
CIZ1	NT_029366:585	NM_012127	149	ZnF-C2H2	Cip1-interacting zinc finger protein
CLOCK		NM_004898	150	bHLH	Clock (mouse) homolog
CNOT3		NM_014516	151	Other	CCRA-NOT transcription complex, subunit 3
CNOT4		NM_013316	152	Other	CCRA-NOT transcription complex, subunit 4
CNOT8		NM_004779	153	Other	CCRA-NOT transcription complex, subunit 8
COPEB		NM_001300	154	ZnF-C2H2	core promoter element binding protein
COPS5		NM_006837	155	Co-activator	COP9 constitutive photomorphogenic homolog subunit 5 (Arabidopsis)
CORO1A		NM_007074	156	bZIP	coronin, actin-binding protein, 1A
CREB1		NM_004379	157	bZIP	cAMP responsive element binding protein 1
CREB3		NM_006468	158	bZIP	cAMP responsive element binding protein 3 (luman)
CREB3L1		NM_052854	159	bZIP	cAMP responsive element binding protein 3-like 1
CREB3L2	NT_007933:5606	NM_194071	160	bZIP	cAMP responsive element binding protein 3-like 2
CREB3L3	NT_011255:184	NM_032607	161	bZIP	cAMP responsive element binding protein 3-like 3
CREB3L4	NT_004858:17	NM_130898	162	bZIP	cAMP responsive element binding protein 3-like 4
CREB5		NM_004904	163	bZIP	cAMP responsive element binding protein 5
CREBBP		NM_004380	164	ZnPHD	CREP binding protein (Rubinstein-Taybi syndrome)
CREBL1		NM_004381	165	bZIP	cAMP responsive element binding protein-like 1
CREBL2		NM_001310	166	bZIP	cAMP responsive element binding protein-like 2
CREG		NM_003851	167	Other	Cellular repressor of EIA-stimulated genes
CREM		NM_001881	168	bZIP	cAMP responsive element

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					modulator
CRIP1		NM_001311	169	Co-activator	cysteine-rich protein 1 (intestinal)
CRIP2		NM_001312	170	Co-activator	cysteine-rich protein 2
CROC4		NM_006365	171	Other	transcriptional activator of the c-fos promoter
CRSP8		NM_004269	172	Co-activator	cofactor required for Sp1 transcriptional activation, subunit 8, 34kD
CRSP9		NM_004270	173	Co-activator	cofactor required for Sp1 transcriptional activation, subunit 9, 33kD
CRX		NM_000554	174	Homeobox	cone-rod homeobox
CSDA		NM_003651	175	Beta-scaffold-cold-shock	cold shock domain protein A
CSEN		NM_013434	176	Other	Calsenilin, presenilin-binding protein, EF hand transcription factor
CSRP1		NM_004078	177	Co-activator	cysteine and glycine-rich protein 1
CSRP2		NM_001321	178	Co-activator	cysteine and glycine-rich protein 2
CSRP3		NM_003476	179	Co-activator	cysteine and glycine-rich protein 3 (cardiac LIM protein)
CTCF		NM_006565	180	ZnF-C2H2	CCCTC-binding factor (zinc finger protein)
CTCFL	NT_011362:1953	NM_080618	181	ZnF-C2H2	CCCTC-binding factor (zinc finger protein)-like
CTNNB1		NM_001904	182	Co-activator	catenin (cadherin-associated protein), beta 1, 88kD
CUTL1		NM_001913	183	Homeobox	cut (Drosophila)-like 1 (CCAAT displacement protein)
CUTL2		AB006631	184	Homeobox	cut-like 2 (Drosophila)-
MAMLD1		NM_001177465.1 NM_001177466.1 NM_005491.3	185 1483 1484	Other	isoform 1 isoform 2 isoform 3
DACH		NM_004392	186	Co-repressor	dachshund (Drosophila) homolog
DAT1		NM_018640	187	ZnF-Other	neuronal specific transcription factor DAT1
DATF1		NM_022105	188	ZnF-PHD	death associated transcription factor 1
DBP		NM_001352	189	bZIP	D site of albumin promoter (albumin D-box) binding protein
DDIT3		NM_004083	190	bZIP	DNA-damage-inducible transcript 3
DEAF1		NM_021008	191	ZnF-MYND	deformed epidermal autoregulatory factor 1 (Drosophila)
DKFZP434 B0335		AB037779	192	Other	DKFZP434B0335 protein
DKFZP434		NM_031284	193	Other	Hypothetical protein

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
B195					DKFZp434B195
DKFZp434 G043		AL080134	194	bIILII	IILIImdelta (fly) homolog
DKFZP434 P1750		NM_015527	195	Other	DKFZP434P1750
DKFZp547 B0714	NT_011233:43	NM_152606	196	ZnF-C2H2	Hypothetical protein DKFZp547B0714
DLX2		NM_004405	197	Homeobox	Distal-less homeobox 2
DLX3		NM_005220	198	Homeobox	distal-less homeobox 3
DLX4		NM_001934	199	Homeobox	distal-less homeobox 4
DLX5		NM_005221	200	Homeobox	distal-less homeobox 5
DLX6		NM_005222	201	Homeobox	distal-less homeobox 6
DMRT1		NM_021951	202	ZnF-DM	doublesex and mab-3 related transcription factor 1
DMRT2		NM_006557	203	ZnF-DM	doublesex and mab-3 related transcription factor 2
DMRT3	NT_008413:158	NM_021240	204	ZnF-DM	doublesex and mab-3 related transcription factor 3
DMRTA1	NT_023974:296	AJ290954	205	ZnF-DM	DMRT-like family A1
DMRTA2		AJ301580	206	ZnF-DM	DMRT-like family A2
DMRTB1	NT_004424:223	NM_033067	207	ZnF-DM	DMRT-like family B with prolien-rich C-terminal, 1
DMRTC1		BC029799	208	ZnF-DM	DMRT-like family C1
DMRTC2	NT_011139:240	NM_033052	209	ZnF-DM	DMRT-like family C2
DMTF1		NM_021145	210	Other	cyclin D binding Nyb-like transcription factor 1
DR1		NM_001938	211	Co-repressor	down-regulator of transcription 1, TBP-binding (negative collector 2)
DRAP1		NM_006442	212	Co repressor	DR1-associated protein 1 (negative cofactor 2 alpha)
DRIL1		NM_005224	213	Structural	dead ringer (Drosophila)-like 1
DRIL2		NM_006465	214	Structural	dead ringer (Drosophila)-like 2 (bright and dead ringer)
DRPLA		NM-001940	215	Co-repressor	dentatorubral-palidoluysian atrophy (atrophin-1)
DSIPI		NM-004089	216	bZIP	delta sleep inducing peptide, immunoreactor
DTX2		AB040961	217	ZnF-other	deltex homolog 2 (Drosophila)
DUX1		NM_012146	218	Homeobox	double homeobox 1
DUX2		NM_012147	219	Homeobox	double homeobox 2
DUX3		NM_012148	220	Homeobox	double homeobox genes 3
DUX4		NM_033178	221	Homeobox	double homeobox 4
DUX5		NM_012149	222	Homeobox	double homeobox 5
DXYS155E		NM_005088	223	Other	DNA segment on chromosome X and Y (unique) 155 expressed sequence

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
E2F1		NM_005225	224	E2F	E2F transcription factor 1
Text cut off					
EED		NM_003797	225	Structural	Embryonic ectoderm development
EGLN1	NT_004753:53	NM_022051	226	ZnF-MYND	egl nine homolog 1 (C. elegans)
EGLN2		NM_017555	227	ZnF-MYND	egl nine homolog 2 (C. elegans)
EGR1		NM_001964	228	ZnF-C2H2	early growth response 1
EGR2		NM_000399	229	ZnF-C2H2	early growth response 2 (Knox-20 (Drosophila) homolog)
EGR3		NM_004430	230	ZnF-C2H2	early growth response 3
EGR4		NM_001965	231	ZnF-C2H2	early growth response 4
EHF		NM_012153	232	Trp cluster-Ets	ets homologous factor
EHZF	NT_011044:150	NM_015461	233	ZnF-PHD	early hematopoietic zinc finger
ELD/OSA1		NM_020732	234	Structural	BRG1-binding protein ELD/OSA1
ELF1		M82882	235	Trp cluster Ets	E-74-like factor 1 (ets domain transcription factor)
ELF2		NM_006874	236	Trp cluster Ets	E-74-like factor 2 (ets domain transcription factor)
ELF3		NM_004433	237	Trp cluster Ets	E-74-like factor 3 (ets domain transcription factor, epithelial-specific)
ELF4		NM_001421	238	Trp cluster Ets	E-74-like factor 4 (ets domain transcription factor)
ELF5		NM_001422	239	Trp cluster Ets	E-74-like factor 5 (ets domain transcription factor)
ELK1		NM_005229	240	Trp cluster Ets	ELK1, member of ETS oncogene family
ELK3		NM_005230	241	Trp cluster Ets	ELK3, ETS-domain protein (SRF accessory protein 2)
ELK4		NM_021795	242	Trp cluster Ets	ELK4, ETS-domain protein (SRF accessory protein 1)
EME2	NT_010552:331	AK074080	243	ZnF-BTB/POZ	essential meiotic endonuclease I homolog 2 (S. pombe)
EMX1		X68879	244	Homeobox	empty spiracles homolog 1 (Drosophila)
EMX2		NM_004098	245	Homeobox	empty spiracles homolog 2 (Drosophila)
EN1		NM_001426	246	Homeobox	engrailed homolog 1
EN2		NM_001427	247	Homeobox	engrailed homolog 2
EC1	NT_oo6713:275	NM_003633	248	ZnF-BTB/POZ	ectodermal-neural cortex (with BTB-like domain)
ENO1		NM_001428	249	Other	enolase 1
EOMES		NM_005442	250	T-box	Eomesodermin (Xenopus

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					laevis) homolog
ERCC3		NM_000122	251	Other	excision repair cross-complementing rodent repair deficiency, complementation group 3
ERCC6		NM_000124	252	Other	excision repair cross-complementing rodent repair deficiency, complementation group 6
ERF		NM_006494	253	Trp cluster-Ets	Ets2 repressor factor
ERG		NM_004449	254	Trp cluster-Ets	v-ets avian erythroblastosis virus E26 oncogene related
ESR1		NM_000125	255	NHR	estrogen receptor 1
ESR2		NM_001437	256	NHR	estrogen receptor 2
ESRRA		NM_004451	257	NHR	estrogen-related receptor alpha
ESRRB		NM_004452	258	NHR	estrogen-related receptor beta
ESRRG		NM_001438	259	NHR	estrogen-related receptor gamma
ESXIL	NT_01165135	NM_153448	260	Homeobox	extraembryonic, spermatogenesis, homeobox 1-like
ETR101		NM_004907	261	Other	immediate early protein
ETS1		NM_005238	262	Trp cluster-Ets	v-ets avian erythroblastosis virus E26 oncogene homolog 1
ETS2		NM_005239	263	Trp cluster-Ets	v-ets avian erythroblastosis virus E26 oncogene homolog 2
ETV1		NM_004956	264	Trp cluster-Ets	ets variant gene 1
ETV2		AF000671	265	Trp cluster-Ets	ets variant gene 2
ETV3		L16464	266	Trp cluster-Ets	ets variant gene3
ETV4		NM_001986	267	Trp cluster-Ets	ets variant gene 4 (E1A enhancer-binding protein, E1AF)
ETV5		NM_004454	268	Trp cluster-Ets	ets variant gene 5 (ets-related molecule)
ETV6		NM_001987	269	Trp cluster-Ets	ets variant gene 6, TEL oncogene
EV11	NT_034563:55	NM_005241	270	ZnF-C2H2	ecotropic viral integration site 1
EVX1		NM_001989	271	Homeobox	eve, even-skipped homeo box homolog 1 (Drosophila)
EVX2		M59983	272	Homeobox	eve, even-skipped homeo box homolog 2 (Drosophila)
EYA1		NM_000503	273	Other	eyes absent (Drosophila) homolog 1
EYA2		NM_005204	274	Other	eyes absent (Drosophila)

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					homolog 2
FBI1		NM_015898	275	ZnF-BTB/POZ	short transcripts binding protein; lymphoma related factor
FEM1A		AL359589	276	Other	fem-1 homolog a (C.elegans)
FEZL		NM_018008	277	ZnF-C2H2	likely ortholog of mouse and zebrafish forebrain embryonic zinc finger-like
FHL1		NM_001449	278	ZnF-Other	four and a half LIM domains 1
FHL2		NM_001450	279	ZnF-Other	four and a half LIM domains 2
FHL5		NM_020482	280	Co-activator	four and a half LIM domains 5
FHX		NM_018416	281	Forkhead	FOXJ2 forkhead factor
FKHL18		AF042831	282	Forkhead	forkhead (Drosophila)-like 18
FLI1		NM_002017	283	Trp cluster-Ets	friend leukemia virus integration 1
FMR2		NM_002025	284	AF-4	fragile X mental retardation 2
FOS		NM_005252	285	bZIP	v-fos FBJ murine osteosarcoma viral oncogene homolog
FOSB		NM_006732	286	bZIP	FBJ murine osteosarcoma viral oncogene homolog B
FOSL1		NM_005438	287	bZIP	FOS-like antigen 1
FOSL2		NM_005253	288	bZIP	FOS-like antigen 2
FOXA1		NM_004496	289	Forkhead	forkhead box A1
FOXA2		NM_021784	290	Forkhead	forehead box A2
FOXE2		NM_012185	291	Forkhead	forkhead box E2
FOXE3		NM_012186	292	Forkhead	forkhead box E3
FOXF1		NM_001451	293	Forkhead	forkhead box F1
FOXF2		NM_001452	294	Forkhead	forkhead box F2
FOXG1B		NM_005249	295	Forkhead	forkhead box G1B
FOXH1		NM_003923	296	Forkhead	forkhead box H1
FOXI1		NM_012188	297	Forkhead	forkhead box I1
FOXJ1		NM_001454	298	Forkhead	forkhead box J1
FOXL1		NM_005250	299	Forkhead	forkhead box L1
FOXL2		NM_023067	300	Forkhead	forkhead box L2
FOXM1		NM_021953	301	Forkhead	forkhead box M1
FOXN4	NT_009770:26	AF425596	302	Forkhead	forkhead/winged helix transcription factor FOXN4
FOXO1A		NM_002015	303	Forkhead	forkhead box O1A (rhabdomyosarcoma)
FOXO3A		NM_001455	304	Forkhead	forkhead box O3A
FOXP1		AF275309	305	Forkhead	forkhead box P1
FOXP2		NM_014491	306	Forkhead	forkhead box P2
FOXP3		NM_014009	307	Forkhead	forkhead box P3
FOXP4	NT_007592:3277	NM_138457	308	Forkhead	forkhead box P4
FOXQ1		NM_033260	309	Forkhead	forkhead box Q1
FREQ	NT_029366:864	NM_014286	310	Other	frequenin homolog (Drosophila)

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
FUBP1		NM_003902	311	Other	far upstream element-binding protein
FUBP3	NT_008338:25	BC001325	312	Other	far upstream element (FUSE) binding protein 3
GABPA		NM_002040	313	Trp cluster-Ets	GA-binding protein transcription factor, alpha subunit (60kD)
GABPB1		NM_005254	314	Co-activator	GA-binding protein transcription factor, beta subunit 1 (53kD)
GABPB2		NM_016655	315	Trp cluster-Ets	GA-binding protein transcription factor, beta subunit 2 (47k D)
GAS41		NM_006530	316	Structural	glioma-amplified sequence-41
GASC1		AB018323	317	ZnF-PHD	gene amplified in squamous cell carcinoma 1
GATA1		NM_002049	318	ZnF-GATA	GATA-binding protein 1 (globin transcription factor 1)
GATA2		NM_002050	319	ZnF-GATA	GATA-binding protein 2
GATA3		NM_002051	320	ZnF-GATA	GATA-binding protein 3
GATA4		NM_002052	321	ZnF-GATA	GATA-binding protein 4
GATA5		NM_080473	322	ZnF-GATA	GATA-binding protein 5
GATA6		NM_005257	323	ZnF-GATA	GATA-binding protein 6
GBX1		L11239	324	Homeobox	gastrulation brain homeobox 1
GBX2		NM_001485	325	Homeobox	gastrulation brain homeobox 2
GFI1		NM_005263	326	ZnF-C2H2	growth factor independent 1
GFI1B		NM_004188	327	ZnF-C2H2	growth factor independent 1B (potential regulator of CDKN1A, translocated in CML)
GIOT-1		AB021641	328	ZnF-C2H2	gonadotropin inducible transcription repressor 1
GIOT-2		NM_016264	329	ZnF-C2H2	gonadotropin inducible transcription repressor-2
GL1		NM_005269	330	ZnF-C2H2	glioma-associated oncogene homolog (zinc finger protein)
GLI2		NM_005270	331	ZnF-C2H2	GLI-Kruppel family member GLI2
GLI3		NM_000168	332	ZnF-C2H2	GLI-Kruppel family member GLI3 (Greig cephalopolysyndactyly syndrome)
GLI4	NT_023684:15	NM_138465	333	ZnF-C2H2	GLI-Kruppel family member GLI4
GLIS2		NM_032575	334	ZnF-C2H2	Kruppel-like zinc finger protein GLIS2
GREB1	NT_005334:553	NM_014668	335	Co-repressor	GREB1 protein
GRLF1		NM_004491	336	ZnF-Other	glucocorticoid receptor DNA binding factor 1

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
GSC		NM_173849.2	337	Homeobox	goosecoid
GSCL		NM_005315	338	Homeobox	goosecoid-like
GSH1		XM_046853	339	Homeobox	genomic screened homeo box 1 homolog (mouse)
GSH2		NM_133267	340	Homeobox	genomic screened homeo box 2 homolog (mouse)
GTF2A1		NM_015859	341	Other	general transcription factor 11A, 1 (37kD and 19kD subunits)
GTF2A2		NM_004492	342	Other	general transcription factor IIA, 2 (12kD subunit)
GTF2B		NM_001514	343	Other	general transcription factor 11B
GTF2E1		NM_005513	344	Other	general transcription factor IIE, polypeptide 1 (alpha subunit, 56kD)
GTF2E2		NM_002095	345	Other	general transcription factor IIE, polypeptide 2 (beta subunit, 34kD)
GTF2F1		NM_002096	346	Other	general transcription factor IIF, polypeptide I (74kD subunit)
GTF2F2		NM_004128	347	Other	general transcription factor IIF, polypeptide 2 (30kD subunit)
GTF2H1		NM_005316	348	Other	general transcription factor IIH, polypeptide I (62kD subunit)
GTF2IRD1	NT_007758:1220	NM_005685	349	bHLH	GTF21 repeat domain containing 1
GTF2IRD2	NT_007758:1320	NM_173537	350	bHLH	transcription factor GTF2IRD2
GTF3A		NM_002097	351	Other	general transcription factor IIIA
GTF3C1		NM_001520	352	Other	general transcription factor IIIC, polypeptide 1 (alpha subunit, 220kD)
GTF3C2		NM_001521	353	Other	general transcription factor IIIC, polypeptide 2 (beta subunit, 110kD)
GTF3C3		NM_012086	354	Other	general transcription factor IIIC, polypeptide 3 (102kD)
GTF3C4		NM_012204	355	Other	general transcription factor IIIC, polypeptide 4 (90kD)
GTF3C5		NM_012087	356	Other	general transcription factor IIIC, polypeptide 5 (63kD)
HAND1		NM_004821	357	bHLH	heart and neural crest derivatives expressed 1
HAND2		NM_021973	358	bHLH	basic helix-loop-helix transcription factor HAND2
HATH6	NT_015805:94	NM_032827	359	bHLH	basic helix-loop-helix transcription factor 6
HBOA		NM_007067	360	Co-activator	histone acetyltransferase
HCF2		NM_013320	361	Other	host cell factor 2

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
HCNGP		NM_013260	362	Other	transcriptional regulator protein
HDAC1		NM_004964	363	Co-repressor	histone deacetylase 1
HDAC2		NM_001527	364	Co-repressor	histone deacetylase 2
HDAC4		NM_006037	365	Co-repressor	histone deacetylase 4
HDAC8	NT-011594:18	NM_018486	366	Structural	histone deacetylase 8
HES2		NM_019089	367	bHLH	hairy and enhancer of split 2 (Drosophila)
HES5		BQ924744	368	bHLH	hairy and enhancer of split 5 (Drosophila)
HES6		NM_018645	369	bHLH	hairy and enhancer of split 6 (Drosophila)
HES7		NM_032580	370	bHLH	hairy and enhancer of split 7 (Drosophila)
HESX1		NM_003865	371	Homeobox	homeobox (expressed in ES cells) 1
HEY1		NM_012258	372	bHLH	hairy/enhancer-of-split related with YRPW motif 1 ('YRPW' disclosed as SEQ ID NO: 1482)
HEY2		NM_012259	373	bHLH	hairy/enhancer-of-split related with YRPW motif 2 ('YRPW' disclosed as SEQ ID NO: 1482)
HEYL		NM_014571	374	bHLH	hairy/enhancer-of-split related with YRPW motif-life ('YRPW' disclosed as SEQ ID NO: 1482)
HHEX		NM_002729	375	Homeobox	hematopoietically expressed homeobox
cutoff					
HIVEP1		NM_002114	376	ZnF-C2H2	human immunodeficiency virus type I enhancer-binding protein 1
HIVEP2		NM_006734	377	ZnF-C2H2	human immunodeficiency virus type I enhancer-binding protein 2
HIVEP3	NT_004852:421	NM_024503	378	ZnF-C2H2	human immunodeficiency virus type 1 enhancer binding protein 3
HKR1		BC004513	379	ZnF-C2H2	GLI-Kruppel family member HKR1
HKR2		M20676	380	ZnF-C2H2	GL1-Kruppel family member HKR2
HKR3		NM_005341	381	ZnF-BTB/POZ	GLI-Kruppel family member HKR3
HLF		NM_002126	382	bZIP	hepatic leukemia factor
HLX1		NM_021958	383	Homeobox	H2.0 (Drosophila)-like homeo box 1
HLXB9		NM_005515	384	Homeobox	homeo box HB9
HMG20A	NT_024654:319	NM_018200	385	Structural	high-mobility group 20A
HMG20B		NM_006339	386	Structural	high-mobility group 20B
HMGA1		NM_002131	387	Beta-scaffold-HMG	high mobility group AT-hook 1
HMGA2		NM_003483	388	Beta-scaffold-HMG	high mobility group AT-hook 2

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
HMGB1		NM_002128	389	Structural	high-mobility group box 1
HMGB2		NM_002129	390	Structural	high-mobility group box 2
HMGB3	NT_011602:55	NM_005342	391	Structural	high-mobility group box 3
HMGN2		NM_005517	392	Structural	high-mobility group nucleosomal binding domain 2
HMX1		NM_018942	393	Homeobox	homeo box (H6 family) 1
HMX2		NM_005519.1	394	Homeobox	homeo box (H6 family) 2
HMX3		XM_114950	395	Homeobox	homeo box (H6 family) 3
HNF4A		NM_000457	396	NHR	hepatocyte nuclear factor 4, alpha
HNF4G		NM_004133	397	NHR	hepatocyte nuclear factor 4, gamma
HOP		NM_032495	398	Homeobox	homeodomain-only protein
HOXA1		NM_005522	399	Homeobox	homeobox A1
HOXA10		NM_018951	400	Homeobox	homeobox A10
HOXA11		NM_005523	401	Homeobox	homeobox A11
HOXA13		NM_000522	402	Homeobox	homeobox A13
HOXA2		NM_006735	403	Homeobox	homeobox A2
HOXA3		NM_030661	404	Homeobox	homeobox A3
HOXA4		NM_002141	405	Homeobox	homeobox A4
HOXA5		NM_019102	406	Homeobox	homeobox A5
HOXB9		NM_024017	407	Homeobox	homeobox B9
HOXC10		NM_017409	408	Homeobox	homeobox C10
HOXC11		NM_014212	409	Homeobox	homeobox C11
HOXC12		X99631	410	Homeobox	homeoboxC12
HOXC13		NM_017410	411	Homeobox	homeoboxC13
HOXC4		NM_014620	412	Homeobox	homeoboxC4
HOXC5		NM_018953	413	Homeobox	homeobox C5
HOXC6		NM_004503	414	Homeobox	homeobox C6
HOXC8		NM_022658	415	Homeobox	homeobox C8
HOXC9		NM_006897	416	Homeobox	homeobox C9
HOXD1		NM_024501	417	Homeobox	homeobox D1
HOXD10		NM_002148	418	Homeobox	homeobox D10
HOXD11		NM_021192	419	Homeobox	homeobox D11
HOXD12		NM_021193	420	Homeobox	homeobox D12
HOXD13		NM_000523	421	Homeobox	homeobox D13
HOXD3		NM_006898	422	Homeobox	homeobox D3
HOXD4		NM_014621	423	Homeobox	homeobox D4
HOXD8		NM_019558	424	Homeobox	homeobox D8
HOXD9		NM_014213	425	Homeobox	homeobox D9
HPCA	NT_00451193	NM_002143	426	Other	hippocalcin
HPCAL1	NT_005334:412	NM_002149	427	Other	hippocalcin-like 1
H-plk		NM_015852	428	ZnF-C2H2	Krueppel-related zinc finger protein
HR		AF039196	429	Jumonji	hairless
HRIHFB2122		NM_007032	430	Other	Tara-like protein (Drosophila)
HRY		NM_005524	431	bHLH	hairy (Drosophila)-homolog
HS747E2A		NM_015370	432	Other	hypothetical protein (RING domain)
HSA275986		NM_018403	433	Other	transcription factor SMIF
HSAJ2425		NM_017532	434	NHR	p65 protein

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
HSF1		NM_005526	435	Heat shock	Heat shock transcription factor 1
HSF2		NM_004506	436	Heat shock	Heat shock transcription factor 2
HSF2BP		NM_007031	437	Co-activator	Heat shock transcription factor 2 binding protein
HSF4		NM_001538	438	Heat shock	Heat shock transcription factor 4
HSFY		NM_033108	439	Heat shock	Heat shock transcription factor, Y-linked
HSGT1		NM_007265	440	Other	suppressor of S. cerevisiae ger2
HSHPX5		X74862	441	Other	HPX-5
HSPC018		NM_014027	442	Other	HSPC018 protein
HSPC059	NT_011233:37	NM_016536	443	ZnF-C2H2	HSPC059 protein
HSPC063	NT_033899:972	NM_014155	444	ZnF-C2H2	HSPC063 protein
HSPC189		NM_016535	445	Other	HSPC189 protein
HSPX153		X76978	446	Homeobox	HPX-153 homeobox
HSRNAFEV	NT_005403:123	NM_017521	447	Trp Cluster-Ets	FEV protein
HSU79252		NM_013298	448	Other	hypothetical protein
ID1		NM_002165	449	bHLH	inhibitor of DNA binding 1, negative helix-loop-helix protein
ID2		NM_002166	450	bHLH	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
ID2B	NT_005999:169	M96843	451	bHLH	inhibitor of DNA binding 2B, dominant negative helix-loop-helix protein
ID3		NM_002167	452	bHLH	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
ID4		NM_001546	453	bHLH	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein
IGHMBP2		NM_002180	454	ZnF-AN1	immunoglobulin mu binding protein 2
ILF1		NM_004514	455	Forkhead	interleukin in enhancer binding factor 1
ILF2		NM_004515	456	ZnF-C2H2	interleukin enhancer binding factor 2, 45 kDa
ILF3		NM_012218	457	ZnF-C2H2	interleukin enhancer binding factor, 3, 90 kDa
INSM1		NM_002196	458	ZnF-C2H2	insulinoma-associated 1
INSM2		NM_032594	459	ZnF-C2H2	insulinoma-associated protein 1A-6
IPF1		NM_000209	460	Homeobox	insulin promoter factor 1, homeodomain transcription factor
IRF1		NM_002198	461	Trp cluster-IRF	interferon regulatory factor 1
IRF2		NM_002199	462	Trp cluster-IRF	interferon regulatory factor 2
IRF3		NM_001571	463	Trp cluster-IRF	interferon regulatory factor 3

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
IRF4		NM_002460	464	Trp cluster-IRF	interferon regulatory factor 4
IRF5		NM_002200	465	Trp cluster-IRF	interferon regulatory factor 5
IRF6		NM_006147	466	Trp cluster-IRF	interferon regulatory factor 6
IRF7		NM_001572	467	Trp cluster-IRF	interferon regulatory factor 7
IRLB		X63417	468	Other	c-myc promoter-binding protein
IRX1		U90307	469	Homeobox	iroquois homeobox protein 1
IRX2		AF319967	470	Homeobox	iroquois homeobox protein 2
IRX3		U90308	471	Homeobox	iroquois homeobox protein 3
IRX4		NM_016358	472	Homeobox	Iroquois homeobox protein 4
IRX5		NM_005853	473	Homeobox	Iroquois homeobox protein 5
IRX6		U90305	474	Homeobox	Iroquois homeobox protein 6
JARID1A	NT_009759:29	NM_005056	475	Jumonji	Jumonji, AT rich interactive domain 1A (RBP2-like)
JARID1B	NT_034408:191	NM_006618	476	Jumonji	Jumonji, AT rich interactive domain 1B (RBP2-like)
JARID1D	NT_011875:152	NM_004653	477	Jumonji	Jumonji, AT rich interactive domain 1D (RBP2-like)
JDP2	NT_026437:1173	NM_130469	478	bZIP	jun dimerization protein 2
JMJ		NM_004973	479	Jumonji	jumonji homolog (mouse)
JMJD1	NT_015805:184	NM_018433	480	Jumonji	jumonji domain containing 1
JMJD2	NT_032971:21	BC002558	481	Jumonji	jumonji domain containing 2
JMJD2B	NT_011255:298	AK026040	482	Jumonji	jumonji domain-containing 2B
JUN		NM_002228	483	bZIP	v-jun avan sarcoma virus 17 oncogene homolog
JUNB		NM_002229	484	bZIP	Jun B proto-oncogene
JUND		NM_005354	485	bZIP	Jun D proto-oncogene
KBTBD10	NT_005332:189	NM_006063	486	ZnF-BTB/POZ	kelch repeat and BTB (POZ) domain containing 10
KBTBD5	NT_005825:210	NM_152393	487	ZnF-BTB/POZ	kelch repeat and BTB (POZ) domain containing 5
KBTBD7	NT_009984:758	NM_032138	488	ZnF-BTB/POZ	kelch repeat and BTB (POZ) domain containing 7
KCNIP1	NT_023132:191	NM_014592	489	Other	Kv channel interacting protein 1
KCNIP2	NT_030059:932	NM_014591	490	Other	Kv channel interacting protein 2

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
KCNIP4	NT_006344:469	NM_025221	491	Other	Kv channel interacting protein 4
KEAP1		NM_012289	492	Other	Kelch-like ECH-associated protein 1
KLF1		NM_006563	493	ZnF-C2H2	Kruppel-like factor 1 (erythroid)
KLF12		NM_007249	494	ZnF-C2H2	Kruppel-like factor 12
KLF13		NM_015995	495	ZnF-C2H2	Kruppel-like factor 13
KLF14		NM_138693	496	ZnF-C2H2	Kruppel-like factor 14
KLF15		NM_014079	497	ZnF-C2H2	Kruppel-like factor 15
KLF16		NM_031918	498	ZnF-C2H2	Kruppel-like factor 16
KLF2		NM_016270	499	ZnF-C2H2	Kruppel-like factor 2 (lung)
KLF3		NM_016531	500	ZnF-C2H2	Kruppel-like factor 3 (basic)
KLF4		NM_004235	501	ZnF-C2H2	Kruppel-like factor 4 (gut)
KLF5		NM_001730	502	ZnF-C2H2	Kruppel-like factor 5 (intestinal)
KLF7		NM_003709	503	ZnF-C2H2	Kruppel-like factor 7 (ubiquitous)
KLF8		NM_007250	504	ZnF-C2H2	Kruppel-like factor 8
KLHL1	NT_024524:413	NM_020866	505	ZnF-BTB/POZ	kelch-like 1 (Drosophila)
KLHL3	NT_016714:116	NM_017415	506	ZnF-BTB/POZ	kelch-like 3 (Drosophila)
KLHL4	NT_011689:82	NM_019117	507	ZnF-BTB/POZ	kelch-like 4 (Drosophila)
KLHL5		NM_015990	508	ZnF-BTB/POZ	kelch-like 5 (Drosophila)
KLHL6	NT_022676:150	NM_130446	509	ZnF-BTB/POZ	kelch-like 6 (Drosophila)
KLHL8	NT_006204:183	NM_020803	510	ZnF-BTB/POZ	kelch-like 8
LDB1		NM_003893	511	Co-activator	LIM domain binding 1
LDB2		NM_001290	512	Co-activator	LIM domain binding 2
LDOC1		NM_012317	513	bZIP	leucine zipper, down-regulated in cancer 1
LEF1		NM_016269	514	Beta-scaffold-HMG	lymphoid enhancer factor 1
LHX1		NM_005568	515	Homeobox	LIM homeobox protein 1
LHX2		NM_004789	516	Homeobox	LIM homeobox protein 2
LHX3		NM_014564	517	Homeobox	LIM homeobox protein 3
LHX4		NM_033343	518	Homeobox	LIM homeobox protein 4
LHX5		NM_022363	519	Homeobox	LIM homeobox protein 5
LHX6		NM_014368	520	Homeobox	LIM homeobox protein 6
LHX8		AB050476	521	Homeobox	LIM homeobox protein 8
LHX9		AJ277915	522	Homeobox	LIM homeobox protein 9
LIM		NM_006457	523	Co-activator	LIM protein (similar to rat protein kinase C-binding enigma)
LIN28		NM_024674	524	Beta-scaffold-cold-shock	RNA-binding protein LIN-28
LISCH7		NM_015925	525	bHLH	liver-specific bHLH-Zip transcription factor
LMO1		NM_002315	526	ZnF-Other	LIM domain only 1 (rhombotin 1)

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LMO2		NM_005574	527	ZnF-Other	LIM domain only 2 (rhombotin-like 1)
LMO4		NM_006769	528	ZnF-Other	LIM domain only 4
LMO6		NM_006150	529	ZnF-Other	LIM domain only 6
LMO7		NM_005358	530	ZnF-Other	LIM domain only 7
LMX1A		AY078398	531	Homeobox	LIM homeobox transcription factor 1, alpha
LMX1B		NM_002316	532	Homeobox	LIM homeobox transcription factor 1, beta
LOC113655		BC011982	533	Other	hypothetical protein BC011982
LOC115468	NT_035560:126a	NM_145326	534	ZnF-C2H2	similar to hypothetical protein FLJ13659
LOC115509	NT_024802:36	NM_138447	535	ZnF-C2H2	hypothetical protein BC014000
LOC115950	NT_011176:403	NM_138783	536	ZnF-C2H2	hypothetical protein BC016816
LOC126295	NT_011255:1	NM_173480	537	ZnF-C2H2	hypothetical protein LOC126295
LOC146542	NT_024802:32a	NM_145271	538	ZnF-C2H2	similar to hypothetical protein MGC13138
LOC148213	NT_033317:111	NM_138286	539	ZnF-C2H2	hypothetical protein FLJ31526
LOC151162		AF055029	540	Other	hypothetical protein LOC151162
LOC283248	NT_033241:294	NM_173587	541	Trp Cluster-Myb	hypothetical protein LOC283248
LOC284346	NT_011109:18	NM_174945	542	ZnF-C2H2	hypothetical protein LOC284346
LOC285346	NT_034534:55	BC014381	543	Methyl-CpG-binding	hypothetical protein LOC285346
LOC286103	NT_031818:174	NM_178535	544	ZnF-C2H2	hypothetical protein LOC286103
LOC51036		NM_015854	545	Other	retinoic acid receptor-beta associated open reading frame
LOC51042		NM_015871	546	ZnF-C2H2	zinc finger protein
LOC51045		NM_015877	547	ZnF-C2H2	Kruppel-associated box protein
LOC51058		NM_015911	548	ZnF-C2H2	hypothetical protein
LOC51123		NM_016096	549	ZnF-C2H2	HSPC038 protein
LOC51186		NM_016303	550	Other	pp21 homolog
LOC51193		NM_016331	551	ZnF-C2H2	zinc finger protein ANC_2H01
LOC51270		NM_016521	552	E2F	E2F-like protein
LOC51290		NM_016570	553	Other	CDA14
LOC51333	NT_024802:6	NM_016643	554	ZnF-C2H2	mesenchymal stem cell protein DSC43
LOC55893		NM_018660	555	ZnF-C2H2	papillomavirus regulatory factor PRF-1
LOC56270		NM_019613	556	Other	hypothetical protein 628
LOC56930		AL365410	557	Other	hypothetical protein from EUROIMAGE 1669387
LOC57209		AJ245587	558	ZnF-C2H2	Kruppel-type zinc finger protein

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LOC57801		NM_021170	559	bHLH	hairy and enhancer of split 4 (Drosophila)
LOC58500		X16282	560	ZnF-C2H2	zinc finger protein (clone 647)
LOC65243		NM_023070	561	ZnF-C2H2	hypothetical protein
LOC86614		NM_033108	562	Heat shock	Heat shock transcription factor 2-like
LOC90322		AK001357	563	ZnF-C2H2	similar to KRAB zinc finger protein KR18
LOC90462		AK027873	564	ZnF-C2H2	similar to Zinc finger protein 84 (Zinc finger protein HPF2)
LOC90589	NT_011176:506	NM_145233	565	bZIP	similar to Zinc finger protein 20 (Zinc finger protein KOX13)
LOC90987		AK000435	566	ZnF-C2H2	similar to ZINC FINGER PROTEIN 184
LOC91120		NM_033196	567	ZnF-C2H2	similar to ZINC FINGER PROTEIN 85 (ZINC FINGER PROTEIN HPF4) (HTF1) (H. sapiens)
LOC91464	NT_011520:1976	AK025181	568	Homeobox	hypothetical protein LOC91464
LOC91614		AJ245600	569	Other	novel 58.3 KDA protein
M96		NM_007358	570	ZnF-PHD	likely ortholog of mouse metal response element binding transcription factor 2
MAD		NM_002357	571	bHLH	MAX dimerization protein 1
MADH1		NM_005900	572	Dwarfin	MAD, mothers against decapentaplegic homolog 1 (Drosophila)
MADH2		NM_005901	573	Dwarfin	MAD, mothers against decapentaplegic homolog 2 (Drosophila)
MADH3		NM_005902	574	Dwarfin	MAD, mothers against decapentaplegic homolog 3 (Drosophila)
MADH4		NM_005359	575	Dwarfin	MAD, mothers against decapentaplegic homolog 4 (Drosophila)
MADH5		NM_005903	576	Dwarfin	MAD, mothers against decapentaplegic homolog 5 (Drosophila)
MADH6		NM_005585	577	Dwarfin	MAD, mothers against decapentaplegic homolog 6 (Drosophila)
MADH7		NM_005904	578	Dwarfin	Mad, mothers against decapentaplegic homolog 7 (Drosophila)
MADH9		NM-005905	579	Dwarfin	MAD, mothers against decapentaplegic homolog 9 (Drosophila)
MAF		NM_005360	580	bZIP	v-maf musculoaponeurotic

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					fibrosarcoma oncogene homolog (avian)
MAFB		NM_005461	581	bZIP	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)
MAFF		NM_012323	582	bZIP	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein F (avian)
MAFG		NM_002359	583	bZIP	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein G (avian)
					v-maf musculoaponeurotic
MBD4		NM_003925	584	Methyl-CpG-binding	methyl-CpG binding domain protein 4
MBNL2		NM_005757	585	ZnF-C3H	muscleblind-like 2 (Drosophila)
MDS032		NM_018467	586	Other	uncharacterized hematopoietic stem/progenitor cells protein MDS032
MDS1		NM_004991	587	Other	myelodysplasia syndrome 1
MECP2		NM_004992	588	Methyl-CpG-binding	methyl CpG binding protein 2 (Rett syndrome)
MED6		NM_005466	589	Co-activator	mediator of RNA polymerase II transcription, subunit 6 homolog (yeast)
MEF2A		NM_005587	590	Beta-scaffold-MADS	MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)
MEF2B		NM_005919	591	Beta-scaffold-MADS	MADS box transcription enhancer factor 2, polypeptide B (myocyte enhancer factor 2B)
MEF2C		NM_002397	592	Beta-scaffold-MADS	MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)
MEF2D		NM_005920	593	Beta-scaffold-MADS	MADS box transcription enhancer factor 2, polypeptide D (myocyte enhancer factor 2D)
MEFV		NM_000243	594	Co-activator	Mediterranean fever (pyrin)
MEIS1		NM_002398	595	Homeobox	Meis1, mycloid ecotropic viral integration site 1 homolog (mouse)
MEIS2		NM_020149	596	Homeobox	Meis1, myeloid ecotropic viral integration site 1 homolog 2 (mouse)
MEIS3		U68385	597	Homeobox	Meis1, myeloid ecotropic viral integration site 1 homolog 3 (mouse)
MEOX1		NM_004527	598	Homeobox	mesenchyme homeobox 1

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
MEOX2		NM_005924	599	Homeobox	mesenchyme homeobox 2 (growth arrest-specific homeo box)
MESP1	NT_033276:146	NM_018670	600	bHLH	mesoderm posterior 1
MESP2		AL360139	601	bHLH	mesoderm posterior 2
METTL3		NM_019852	602	Other	methyltransferase like 3
MGA		AB011090	603	bHLH	MAX gene associated
MHC2TA		NM_000246	604	Other	MHC class II transactivator
MID1		NM_000381	605	Structural	midline 1 (Opitz/BBB syndrome)
MID2	NT_011651:146	NM_012216	606	Structural	midline 2
MI-ER1		NM_020948	607	Other	mesoderm induction early response 1
MILD1		NM_031944	608	Homeobox	Mix1 homeobox-like 1 (Xenopus laevis)
MITF		NM_000248	609	bHLH	microphthalmia-associated transcription factor
MLLT1		NM_005934	610	AF-4	myeloid/lymphoid or mixed-lineage leukemia (thrithorax (Drosophila) homolog); translocated to, 1
MLLT10		NM_004641	611	ZnF-PHD	myeloid/lymphoid or mixed-lineage keukemia (trithorax (Drosophila) homolog); translocated to 10
MLLT2		NM_005935	612	AF-4	myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to 2
MLLT3		NM_004529	613	AF-4	myleloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to 3
MLLT4		NM_005936	614	Structural	myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to 4
MLLT6		NM_005937	615	ZnF-PHD	myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to 6
MLLT7		NM_005938	616	Forkhead	myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 7
MNAT1		NM_002431	617	ZnF-Other	menage a trois 1 (CAK assembly factor)
MNDA		NM_002432	618	Other	myeloid cell nuclear

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					differentiation antigen
MNT		NM_020310	619	bHLH	MAX binding protein
MONDOA		NM_014938	620	bHLH	Mix interactor
MORF		NM_012330	621	ZnF-PHD	monocytic leukemia zinc finger protein-related facto
MORF4		NM_006792	622	Structural	mortality factor 4
MORF4L1		NM_006791	623	Structural	mortality factor 4 like 1
MORF4L2		NM_012286	624	Structural	mortality factor 4 like 2
MRF-1		BC032488	625	Structural	modulator recognition factor 1
MRF2		BC015120	626	Structural	modulator recognition factor 2
MRG2		AL359938	627	Homeobox	likely ortholog of mouse myeloid ecotropic viral integration site-related gene 2
MTF1		NM_005955	628	ZnF-C2H2	[cut off] transcription factor 1
MXD3		NM_031300	629	bHLH	MAX dimerization protein 3
MXD4		NM_006454	630	bHLH	MAX dimerization protein 4
MXI1		NM_005962	631	bHLH	MAX interacting protein 1
MYB		NM_005375	632	Trp cluster-Myb.	v-myb myeloblastosis viral oncogene homolog (avian)
MYBBP1A		NM_014520	633	Co-repressor	MYB binding protein (P160) 1a
MYBL1		X66087	634	Trp cluster-Myb	v-myb myeloblastosis viral oncogene homolog (avian)-like 1
MYBL2		NM_002466	635	Trp cluster-Myb	v-myb myeloblastosis viral oncogene homolog (avian)-like 2
MYC (c-MYC)		NM_002467	636	bHLH	v-myc myelocytomatosis viral oncogene homolog (avian)
MYCBP		NM_012333	637	Co-activator	c-myc binding protein
MYCL1		M19720	638	bHLH	v-myc myelocytomatosis viral oncogene homolog , lung carcinoma derived (arivan)
MYCL2		NM_005377	639	bHLH	v-myc myelocytomatosis viral oncogene homolog 2 (avian)
MYCLK1		M64786	640	bHLH	v-myc myelocytomatosis viral oncogene homolog (avian)-like 1
MYCN		NM_005378	641	bHLH	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
MYF5		NM_005593	642	bHLH	myogenic factor 5
MYF6		NM_002469	643	bHLH	myogenic factor 6 (herculin)
MYNN	NT_010840:25	NM_018657	644	ZnF-	myoneurin

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
				BTB/POZ	
MYOD1		NM_002478	645	bHLH	myogenic factor 3
MYOG		NM_002479	646	bHLH	myogenin (myogenic factor 4)
MYT1		NM_004535	647	ZnF-Other	myelin transcription factor 1
MYT1L		AF036943	648	ZnF-Other	myelin transcription factor 1-like
MYT2		NM_003871	649	Other	myelin transcription factor 2
NAB1		NM_005966	650	Co-repressor	NGFI-A binding protein 1 (EGR1 binding protein 1)
NAB2		NM_005967	651	Co-repressor	NGFI-A binding protein 2 (EGR1 binding protein 2)
NCALD		NM_032041	652	Other	neurocalcin delta
NCOA1		NM_003743	653	Co-activator	nuclear receptor
NCYM		NM_006316	654	Other	transcriptional activator
NEUD4		NM_004647	655	ZnF-PHD	Neuro-d4 (rat) homolog
NEUROD1		NM_002500	656	bHLH	neurogenic differentiation 1
NEUROD2		NM_006160	657	bHLH	neurogenic differentiation 2
NEUROD4		NM_021191	658	bHLH	neurogenic differentiation 4
NEUROD6		NM_022728	659	bHLH	neurogenic differentiation 6
NEUROG1		NM_006161	660	bHLH	neurogenin 1
NEUROG2		AF303002	661	bHLH	neurogenin 2
NEUROG3		NM_020999	662	bHLH	neurogenin 3
NFAT5		NM_006599	663	Beta-scaffold-RHD	nuclear factor of activated T-cells 5, tonicity-responsive
NFATC1		NM_006162	664	Beta-scaffold-RHD	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
NFATC2		NM_012340	665	Beta-scaffold-RHD	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2
NFATC3		NM_004555	666	Beta-scaffold-RHD	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
NFATC4		NM_004554	667	Beta-scaffold-RHD	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4
NFE2		NM_006163	668	bZIP	nuclear factor (erythroid-derived 2), 45kD
NFE2L1		NM_003204	669	bZIP	nuclear factor (erythroid-derived 2)-like 1
NFE2L2		NM_006164	670	bZIP	nuclear factor (erythroid-derived 2)-like 2
NFE2L3		NM_004289	671	bZIP	nuclear factor (erythroid-derived 2)-like 3
NFIA		AB037860	672	Beta-scaffold-CCAAT	nuclear factor I/A
NFIB		NM_005596	673	Beta-scaffold-CCAAT	nuclear factor I/B

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NFIC		NM_005597	674	Beta-scaffold-CCAAT	nuclear factor I/C (CCAAT-binding transcription factor)
NFIL3		NM_005384	675	bZIP	nuclear factor, interleukin 3 regulated
NFIX		NM_002501	676	Beta-scaffold-CCAAT	nuclear factor I/X (CCAAT-binding transcription factor)
NFKBIB		NM_002503	677	Co-activator	kappa light polypeptide gene enhancer in B-cells inhibitor, beta
NFKBIE		NM_004556	678	Co-repressor	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon
NFKBIL1		NM_005007	679	Co-repressor	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1
NFKBIL2		NM_013432	680	Co-repressor	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 2
NFRKB		NM_006165	681	Beta-scaffold-RHD	nuclear factor related to kappa B binding protein
NFX1		NM_002504	682	RFX	nuclear transcription factor, X-box binding 1
NFYA		NM_002505	683	Beta-scaffold-CCAAT	nuclear transcription factor Y, alpha
NFYB		NM_006166	684	Beta-scaffold-CCAAT	nuclear transcription factor Y, beta
NFYC		NM_014223	685	Beta-scaffold-CCAAT	nuclear transcription factor Y, gamma
NHLH1	NT_004982:183	NM_005598	686	bHLH	nescient helix loop helix 1
NHLH2		NM_005599	687	bHLH	nescient helix loop helix 2
NKX2-2		NM_002509	688	Homeobox	NK2 transcription factor related, locus 2 (Drosophila)
NKX2-3		NM_145285	689	Homeobox	NK2 transcription factor related, locus 3 (Drosophila)
NKX2-4		AF202037	690	Homeobox	NK2 transcription factor related, locus 4 (Drosophila)
NKX2-5		NM_004387	691	Homeobox	NK2 transcription factor related, locus 5 (Drosophila)
NKX2-8		NM_014360	692	Homeobox	NK2 transcription factor related, locus 8 (Drosophila)
NKX3-1		NM_006167	693	Homeobox	NK3 transcription factor related, locus 1 (Drosophila)
NKX6-1		NM_006168	694	Homeobox	NK6 transcription factor related, locus 1 (Drosophila)

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
NKX6-2		NM_177400	695	Homeobox	NK6 transcription factor related, locus 2 (Drosophila)
NM1		NM_004688	696	Co-activator	N-myc (and STAT) interactor
NPAS1		NM_002517	697	bHLH	neuronal PAS domain protein 1
NR1D2		NM_005126	698	NHR	subfamily 1, group D, member 2
NR1H2		NM_007121	699	NHR	nuclear receptor subfamily 1, group H, member 2
NR1H3		NM_005693	700	NHR	nuclear receptor subfamily 1, group H, member 3
NR1H4		NM_005123	701	NHR	nuclear receptor subfamily 1, group H, member 4
NR1I2		NM_003889.3 NM_022002.2 NM_033013.2	702 1485 1486	NHR	nuclear receptor subfamily 1, group I, member 2 (isoforms 1-3)
NRI13		NM_005122	703	NHR	nuclear receptor subfamily 1, group I, member 3
NR2C1		NM_003297	704	NHR	nuclear receptor subfamily 2, group C, member 1
NR2C2		NM_003298	705	NHR	nuclear receptor subfamily 2, group C, member 2
NR2E1		NM_003269	706	NHR	nuclear receptor subfamily 2, group E, member 1
NR2E3		NM_016346	707	NHR	nuclear receptor subfamily 2, group E, member 3
NR2F1		NM_005654	708	NHR	nuclear receptor subfamily 2, group F, member 1
NR2F2		NM_021005	709	NHR	nuclear receptor subfamily 2, group F, member 2
NR2F6		NM_005234	710	NHR	nuclear receptor subfamily 2, group F, member 6
NR3C1		NM_000176	711	NHR	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
NR3C2		NM_000901	712	NHR	nuclear receptor subfamily 3, group C, member 2
NR4A1		NM_002135	713	NHR	nuclear receptor subfamily 4, group A, member 1
NR4A2		NM_006186	714	NHR	nuclear receptor subfamily 4, group A, member 2
NR4A3		NM_006981	715	NHR	nuclear receptor subfamily 4, group A, member 3
NR5A1		NM_004959	716	NHR	nuclear receptor subfamily 5, group A, member 1
NR5A2		NM_003822	717	NHR	nuclear receptor subfamily 5, group A, member 2
NR6A1		NM_001489	718	NHR	nuclear receptor subfamily 6, group A, member 1
NRF		NM_017544	719	Other	transcription factor
OG2x		AC004534	720	Homeobox	homeobox (mouse) homolog
OLIG1		BC026989	721	bHLH	oligodendrocyte transcription factor 1

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
OLIG2		NM_005806	722	bHLH	oligodendrocyte transcription factor 2
OLIG3		NM_175747	723	bHLH	oligodendrocyte transcription factor 3
ONECUT1		U96173	724	Homeobox	one cut domain, family member 1
ONECUT2		NM-004852	725	Homeobox	one cut domain, family member 2
OPTN		NM_021980	726	Co-activator	optineurin
OSR1		NM_145260	727	ZnF-C2H2	odd-skipped related 1
OSR2	NT_008046:515	NM_053001	728	ZnF-C2H2	odd-skipped-related 2A protein
OTEX	NT_011588:87	NM_139282	729	Homeobox	paired-like homeobox protein OTEX
OTP	NT_006713:546	NM_032109	730	Homeobox	orthopedia homolog (Drosophila)
OTX1		NM_014562	731	Homeobox	orthodenticle homolog 1 (Drosophila)
OTX2		NM_021728	732	Homeobox	orthodenticle homolog 2 (Drosophila)
OTX3		NM_147192	733	Homeobox	orthodenticle homolog 3 (Drosophila)
OVOL1		NM_004561	734	ZnF-C2H2	ovo-like 1(Drosophila)
OVOL3		AD001527	735	ZnF-C2H2	ovo-like 3 (Drosophila)
p100		NM_014390	736	Co-activator	EBNA-2 Co-activator (100kD)
P1P373C6		NM_019110	737	ZnF-C2H2	hypothetical protein P1 p373c6
P381IP		NM_017569	738	Other	transcription factor (p38 interacting protein)
PAWR	NT_019546:106	NM_002583	739	bZIP	PRKC, apoptosis, WT1, regulator
PAX1		NM_006192	740	Paired Box	paired box gene 1
PAX2		NM_000278	741	Paired Box	paired box gene 2
PAX3		NM_000438	742	Paired Box	paired box gene 3 (Waardenburg syndrome 1)
PAX4		NM_006193	743	Paired Box	paired box gene 4
PAX5		NM_016734	744	Paired Box	paired box gene 6 (B-cell lineage specific activator protein)
PAX6		NM_000280	745	Paired Box	paired box gene 6 (aniridia, keratitis)
PAX7		NM_002584	746	Paired box	paired box gene 7
PAX8		NM_003466	747	Paired Box	paired box gene 8
PAX9		NM_006194	748	Paired Box	paired box gene 9
PAXIP1L		U80735	749	Co-activator	PAX transcription activation domain interacting protein 1 like
PBX1		NM_002585	750	Homeobox	pre-B-cell leukemia transcription factor 1
PBX2		NM_002586	751	Homeobox	pre-B-cell leukemia transcription factor 2
					glutamine/Q-rich-associated protein
PDEF		NM_012391	752	Trp cluster-	prostate epithelium-

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
				Ets	specific Ets transcription factor
PEGASUS		NM_022466	753	ZnF-C2H2	zinc finger protein, subfamily 1A, 5 (Pegasus)
PER1		NM_002616	754	bHLH	period homolog 1 (Drosophila)
PER2		NM_003894	755	bHLH	period homolog 2 (Drosophila)
PER3		NM_016831	756	bHLH	period homolog 3 (Drosophila)
PFDN5		NM_002624	757	Co-repressor	prefoldin 5
PGR		NM_000926	758	NHR	progesterone receptor
PHC1		NM_004426	759	Structural	polyhomeotic-like 1 (Drosophila)
PHD3		NM_015153	760	ZnF-PHD	PHD finger protein 3
PHF15	NT_034776:94	NM_015288	761	ZnF-PHD	PHD finger protein 15
PHF16	NT_011568:120	NM_014735	762	ZnF-PHD	PHD finger protein 6
PHTF1		NM_006608	763	Homeobox	putative homeodomain transcription factor
PIAS1	NT_010222:2	NM_016166	764	ZnF-MIZ	protein inhibitor of activated STAT, 1
PIAS3		NM_006099	765	ZnF-MIZ	protein inhibitor of activated STAT3
PIASY	NT_011255:153	NM_015897	766	ZnF-MIZ	protein inhibitor of activated STAT protein PIASy
PIG7		NM_004862	767	Other	LPS-induced TNF-alpha factor
PILB		NM_012228	768	Other	pilin-like transcription factor
PITX1		NM_002653	769	Homeobox	paired-like homeodomain transcription factor 1
PITX2		NM_000325	770	Homeobox	paired-like homeodomain transcription factor 2
PITX3		NM_005029	771	Homeobox	paired-like homeodomain transcription factor 3
PKNOX1		NM_004571	772	Homeobox	PBX/knotted 1 homeobox 1
PKNOX2		NM_022062	773	Homeobox	PBX/knotted 1 homeobox 2
PLAG1		NM_002655	774	ZnF-C2H2	pleiomorphic adenoma gene 1
PLGAL1		NM_002656	775	ZnF-C2H2	pleiomorphic adenoma gene-like 1
PLAGL2		NM_002657	776	ZnF-C2H2	pleiomorphic adenoma gene-like 2
PLRG1		NM_002669	777	Co-repressor	pleiotropic regulator 1 (PRL1 homolog, Arabidopsis)
PMF1		NM_007221	778	Co-activator	polyamine-modulated factor 1
PML		NM_002675	779	Structural	promyelocytic leukemia
PMX1		NM_006902	780	Homeobox	paired mesoderm homeo box 1
POU3F1		NM_002699	781	Homeobox	POU domain, class 3, transcription factor 1

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POU3F2		NM_005604	782	Homeobox	POU domain, class 3, transcription factor 2
POU3F3		NM_006236	783	Homeobox	POU domain, class 3, transcription factor 3
POU3F4		NM_000307	784	Homeobox	POU domain, class 3, transcription factor 4
POU4F1		NM_006237	785	Homeobox	POU domain, class 4, transcription factor 1
POU4F2		NM_004575	786	Homeobox	POU domain, class 4, transcription factor 2
POU4F3		NM_002700	787	Homeobox	POU domain, class 4, transcription factor 3
POU5F1 (OCT4)		NM_002701	788	Homeobox	POU domain, class 5, transcription factor 1
POU6F1		NM_002702	789	Homeobox	POU domain, class 6, transcription factor 1
PPARA		NM_005036	790	NHR	peroxisome proliferative activated receptor, alpha
PPARBP		NM_004774	791	Co-activator	peroxisome proliferator activated receptor binding protein
PPARD		NM_006238	792	NHR	peroxisome proliferative activated receptor, delta
PPARG		NM_005037	793	NHR	peroxisome proliferative activated receptor, gamma
PPARGC1		NM_013261	794	Co-activator	peroxisome proliferative activated receptor, gamma, coactivator 1
PRDM1		NM_001198	795	Structural	PR domain containing 1, with ZNF domain
PRDM10		NM_020228	796	Structural	PR domain containing 10
PRDM11		NM_020229	797	Structural	PR domain containing 11
PRDM12		NM_021619	798	Structural	PR domain containing 12
PRDM13		NM_021620	799	Structural	PR domain containing 13
PRDM14		NM_024504	800	Structural	PR domain containing 14
PRDM15		NM_144771	801	Structural	PR domain containing 15
PRDM16		NM_022114	802	Structural	PR domain containing 16
PRDM2		NM_012231	803	Structural	PR domain containing 2, with ZNF domain
PRDM4		NM_012406	804	Structural	PR domain containing 4
PRDM5		NM_018699	805	Structural	PR domain containing 5
PRDM6		AF272898	806	Structural	PR domain containing 6
PRDM7		AF274348	807	Structural	PR domain containing 7
PRDM8		NM_020226	808	Structural	PR domain containing 8
PROX1		NM_002763.3	809	Homeobox	homeobox 1
PRX2		NM_016307	810	Homeobox	paired related homeobox protein
PSIP1		NM_021144.3 NM_001128217.1 NM_033222.3	811 1487 1488	Co-activator	PC4 and SFRS1 interacting protein 1 (isoforms 1-3)
PSMC2	NT_007933:2739	NM_002803	812	Co-activator	proteasome (prosome, macropain) 26S subunit, ATPase, 2
PSMC5		NM_002805	813	Co-activator	proteasomes (prosome, macropain) 26S subunit, ATPase, 5

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PTF1A	NT_008705:1995	NM_178161	814	bHLH	pancreas specific transcription factor, 1a
PTTG1IP		NM_004339	815	Co-activator	pituitary tumor-transforming 1 interacting protein
PURA		NM_005859	816	Other	purine-rich element binding protein A
R28830_2		AC003682	817	ZnF-Other	similar to ZNF197 (ZNF20)
R32184_3		NM_033420	818	Other	hypothetical protein MGC4022
RAI		NM_006663	819	Co-repressor	RelA-associated inhibitor
RAI15		U50383	820	Other	retinoic acid induced 15
RAA		NM_000964	821	NHR	retinoic acid receptor, alpha
RARB		NM_000965	822	NHR	retinoic acid receptor, beta
RARG		NM_000966.4 NM_001042728.1	823 1489	NHR	retinoic acid receptor, gamma (isoforms 1-2)
RAX		NM_013435	824	Homeobox	retina and anterior neural fold homeobox
RB1		NM_000321	825	Pocket domain	retinoblastoma 1 (including osteosarcoma)
RBAF600		AB007931	826	ZnF-C2H2	retinoblastoma-associated factor 600
RBAK	NT_007819:532	NM_021163	827	Other	RB-associated KRAB repressor
RBBP5		NM_005057	828	Co-repressor	retinoblastoma binding protein 5
RBBP9		NM_006606	829	Co-repressor	retinoblastoma binding protein 9
RBL1		NM_002895	830	Pocket domain	retinoblastoma-like 1 (p107)
RBL2		NM_005611	831	Pocket domain	retinoblastoma-like 2 (p130)
RBPSUH		NM_016270	832	ZnF-C2H2	recombining binding protein suppressor of hairless (Drosophila)
RBPSUHL		NM_014276	833	Other	recombining binding protein suppressor of hairless-like (Drosophila)
RCOR		NM_015156	834	Other	REST corepressor
RCV1		NM_002903	835	Other	recoverin
REL		NM_002908	836	Beta-scaffold-RHD	v-rel reticuloendotheliosis viral oncogene
					(avian)
REQ		NM_006268	837	ZnF-PHD	requiem, apoptosis response zinc finger gene
RERE		NM_012102	838	Other	arginine-glutamic acid dipeptide (RE) repeats
REST		NM_005612	839	ZnF-C2H2	RE1-silencing transcription factor
TRIM27	NT_033168:4	NM_006510.4	840	Structural	tripartite motif containing 27
TRIM13		NM_005798.3 NM_052811.2 NM_213590.1	841 1499 1500	Structural	tripartite motif containing 13 (isoforms 1, 1, 1, and 2, respectively)

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		NM_001007278.1	136		
RFPL3	NT_011520:1735	NM_006604	842	Structural	ret finger protein-like 3
RFX1		NM_002918	843	RFX	regulatory factor X, 1 (influences HLA class II expression)
RFX2		NM_000635	844	RFX	regulatory factor X, 2 (influences HLA class II expression)
RFX3		NM_002919	845	RFX	regulatory factor X, 3 (influences HLA class II expression)
RFX4		NM_002920	846	RFX	regulatory factor X, 4 (influences HLA class II expression)
RFX5		NM_000449	847	RFX	regulatory factor X, 5 (influences HLA class II expression)
RFXANK		NM_003721	848	Co-activator	regulatory factor X-associated ankyrin-containing protein
RGC32		NM_014059	849	Other	RGC32 protein
RIN3	NT_026437:2459	NM_024832	850	bHLH	Ras and Rab interactor 3
RING1		NM_002931	851	ZnF-Other	ring finger protein 1
RIP60		NM_013400	852	ZnF-C2H2	replication initiation region protein (60kD)
RIPX	NT_006216:11	NM_014961	853	ZnF-PHD	rap2 interacting protein x
RLF		NM_012421	854	ZnF-C2H2	rearranged L-myc fusion sequence
RNF10		NM_014868	855	ZnF-Other	ring finger protein 10
RNF12		NM_016120	856	ZnF-Other	ring finger protein 12
RNF 13		NM_007282	857	ZnF-Other	ring finger protein 13
RNF135	NT_035420:144	NM_032322	858	ZnF-MIZ	ring finger protein 135 isoform 1
RNF137	NT_028310:82	NM_018073	859	Structural	ring finger protein 137
RNF14		NM_004290	860	Co-activator	ring finger protein 14
RNF144		NM_014746	861	ZnF-Other	Ring finger protein 144
RNF18	NT_033240:76	NM_020358	862	Structural	ring finger protein 18
RNF2		NM_007212	863	Co-repressor	ring finger protein 2
RNF24		NM_007219	864	ZnF-Other	ring finger protein 24
RNF3		NM_006315	865	ZnF-Other	ring finger protein 3
RNF36	NT_0101942	NM_080745	866	Structural	ring finger protein 36
RNF4		NM_002938	867	ZnF-Other	ring finger protein 4
RNF8		NM_003958	868	ZnF-Other	ring finger protein (C3HC4 type) 8
RORA		NM_134261.2 NM_134260.2 NM_002943.3 NM_134262.2	869 1490 1491 1492	____	RAR-related orphan receptor A (isoforms a-d)
RORB		NM_006914.3	1493		RAR-related orphan receptor B
RORC		NM_005060.3 NM_001001523.1	1494 1495		RAR-related orphan receptor C (isoforms a-b)
RUNX1		NM_001754	870	scaffold-RUNT	(acute myeloid leukemia 1; aml1 oncogene)
RUNX2		NM_004348	871	Beta-scaffold-	runt-related transcription

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
				RUNT	factor 2
RUNX3		NM_004350	872	Beta-scaffold-RUNT	runt-related transcription factor 3
RXRA		NM_002957	873	NHR	retinoid X receptor, alpha
RXRB		NM_021976	874	NHR	retinoid X receptor, beta
RXRG		NM_006917	875	NHR	retinoid X receptor, gamma
RYBP	NT_005526:6	NM_012234	876	Co-repressor	RING1 and YY1 binding protein
SAFB		NM_002967	877	Other	scaffold attachment factor B
SALL1		NM_002968	878	ZnF-C2H2	sal-like 1 (Drosophila)
SALL2		AB002358	879	ZnF-C2H2	sal-like 2 (Drosophila)
SALL3		NM_171999	880	ZnF-C2H2	sal-like 3 (Drosophila)
SALL4		NM_020436	881	ZnF-C2H2	similar to SALL1 (sal (Drosophila)-like
SAP18		NM_005870	882	Co-repressor	sin3-associated polypeptide, 18kD
SAP30		NM_003864	883	Co-repressor	sin3-associated polypeptide, 30kD
SART3		NM_014706	884	Co-activator	squamous cell carcinoma antigen recognized by T cells 3
SATB1		NM_002971	885	Homeobox	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNAs)
SATB2	NT_005037:10	NM_015265	886	Homeobox	SATB family member 2
SBB103		NM_005785	887	ZnF-Other	hypothetical SBB103 protein
SBLF		NM_006873	888	Other	stoned B-like factor
SBZF3	NT_031730:7	NM_020394	889	ZnF-C2H2	zinc finger protein SBZF3
SCA2		NM_002973	890	Other	spinocerebellar ataxia 2 (Olivopontocerebellar ataxia 2, autosomal dominant, ataxin 2)
SCAND1		NM_016558	891	Co-activator	SCAN domain-containing 1
SCAND2		NM_022050	892	Co-activator	SCAN domain-containing 2
SCMH1	NT_004852:374	NM_012236	893	Structural	sex comb on midleg homolog 1 (Drosophila)
SCML1		NM_006746	894	Structural	sex comb on midleg-like 1 (Drosophila)
SCML2		NM_006089	895	Structural	sex comb on midleg-like 2 (Drosophila)
SCML4	NT_033944:303	NM_198081	896	Trp Cluster-Ets	sex comb on midleg-like 4
SETDB1		NM_012432	897	Structural	[cut off] bifurcated 1
SF1		NM_004630	898	ZnF-Other	splicing factor 1
SHARP		NM_015001	899	Co-repressor	SMART/HDAC1 associated repressor protein
SHOX		NM_000451.3 NM_006883.2	900 1496	Homeobox	short stature homeobox (isoforms a-b)
SHOX2		NM_003030	901	Homeobox	short stature homeobox 2

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SIAH1		NM_003031	902	Co-repressor	seven in absentia homolog 1 (Drosophila)
SIAH2		NM_005067	903	Co-repressor	seven in absentia homolog 2 (Drosophila)
SIM1		NM_005068	904	bHLH	single-minded homolog 1 (Drosophila)
SIM2		NM_005069	905	bHLH	single-minded homolog 2 (Drosophila)
SIN3B		AB014600	906	Co-activator	SIN3 homolog B, transcriptional regulator (yeast)
SIX1		NM_005982	907	Homeobox	sine oculis homeobox homolog 1 (Drosophila)
SIX2		NM_016932	908	Homeobox	sine oculis homeobox homolog 2 (Drosophila)
SIX3		NM_005413	909	Homeobox	sine oculis homeobox homolog 3 (Drosophila)
SIX4		NM_017420	910	Homeobox	sine oculis homeobox homolog 4 (Drosophila)
SIX5		X84813	911	Homeobox	sine oculis homeobox homolog 5 (Drosophila)
SIX6		NM_007374	912	Homeobox	sine oculis homeobox homolog 6 (Drosophila)
SLB		AL110218	913	Co-repressor	selective LIM binding factor
SLC2A4RG	NT_011333:173	NM_020062	914	ZnF-C2H2	SLC2A4 regulator
SMARCA1		NM_003069	915	Structural	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1
SMARCA2		NM_003070	916	Structural	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2
SMARCA3		NM_003071	917	Structural	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 3
SMARCA4		NM_003072	918	Structural	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4
					subfamily a-like 1
SMARCB1		NM_003073	919	Other	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1
SMARCC1		NM_003074	920	Structural	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c,

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					member 1
SMARCC2		NM_003075	921	Structural	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2
SMARCE1		NM_003079	922	Structural	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1
KDM5C		NM_004187.3 NM_001146702.1	923 924	Structural	lysine (K)-specific demethylase 5C
SNAI1		NM_005985	925	ZnF-C2H2	snail homolog 1 (Drosophila)
SNAI2		NM_003068	926	ZnF-C2H2	snail homolog 2 (Drosophila)
SNAI3		BC041461	927	ZnF-C2H2	snail homolog 3 (Drosophila)
SNAPC1		NM_003082	928	Other	small nuclear RNA activating complex, polypeptide 1, 43kDa
SNAPC2		NM_003083	929	Other	small nuclear RNA activating complex, polypeptide 2, 45kDa
SNAPC3		NM_003084	930	Other	small nuclear RNA activating complex, polypeptide 3, 50kDa
SNAPC4		NM_003086	931	Other	small nuclear RNA activating complex, polypeptide 4, 190kDa
SNAPC5		NM_006049	932	Other	small nuclear RNA activating complex, polypeptide 5, 19kDa
SNFT		NM_018664	933	bZIP	Jun dimerization protein p21SNFT
SNW1		NM_012245	934	Co-activator	SKI-interacting protein
SOLH	NT_010552:127	NM_005632	935	ZnF-PHD	small optic lobes homolog (Drosophila)
SOM	NT_004391:39	NM_021180	936	Beta-scaffold-grainyhead	sister of mammalian grainyhead
SOX1		NM_005986	937	Beta-scaffold-HMG	SRY (sex determining region Y)-box 1
SOX10		NM_006941	938	Beta-scaffold-HMG	SRY (sex determining region Y)-box 10
SOX11		NM_003108	939	Beta-scaffold-HMG	SRY (sex determining region Y)-box 11
SOX18		NM_018419.2	940	Beta-scaffold-HMG	SRY (sex determining region Y)-box 18
SOX2		L07335	941	Beta-scaffold-HMG Seed SRY	(sex determining region Y)-box 2
SOX21		NM_007084	942	Beta-Scaffold – HMG	SRY (Sex determining region Y)-box 21
SOX3		NM_005634	943	Beta-Scaffold-	SRY (sex determining region Y)-box 3

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
				HMG	
SOX30		NM_007017	944	Beta-scaffold-HMG	SRY (sex determining region Y)-box 30
SOX4		NM_003107 6659	945	Beta-scaffold-HMG	SRY (sex determining region Y)-box 4
SOX5		NM_006940 6660	946	Beta-scaffold-HMG	SRY (sex determining region Y)-box 5
SOX6		NM_033326 55553	947	Beta-scaffold-HMG	SRY (sex determining region Y)-box 6
SOX7	NT_008010:24	NM_031439	948	Beta-scaffold-HMG	SRY (sex determining region Y)-box 7
SOX8		NM_014587 30812	949	Beta-scaffold-HMG	SRY (sex determining region Y)-box 8
SOX9		NM_000346 6662	950	Beta-scaffold-HMG	SRY (sex determining region Y)-box 9
SP1		J03133	951	ZnF-C2H2	Sp1 transcription factor
SP100	NT_005403:864	NM_003113	952	Beta-scaffold-HMG	nuclear antigen Sp100
SP2		NM_003110	953	ZnF-C2H2	Sp2 transcription factor
SP3		X68560	954	ZnF-C2H2	Sp3 transcription factor
SP4		NM_003112	955	ZnF-C2H2	Sp4 transcription factor
SP7	NT_009563:27	NM_152860	956	ZnF-C2H2	Sp7 transcription factor
SPI1		NM_003120	957	Trp cluster-Ets	spleen focus forming virus (SFFV) proviral integration oncogene spi1
SPIB		NM_003121	958	Trp cluster-Ets	Spi-B transcription factor (Spi-1/PU.1 related)
SPIC	NT_009743:37	NM_152323	959	Trp Cluster-Ets	likely ortholog of mouse Spi-C transcription factor (Spi-1/PU.1 related)
SRA1		AF293024	960	Co-activator	steroid receptor RNA activator 1
SRCAP		NM_006662	961	Structural	Snf2-related CBP activator protein
SREBF1		NM_004176	962	bHLH	sterol regulatory element binding transcription factor 1
SREBF2		NM_004599	963	bHLH	sterol regulatory element binding transcription factor 2
SRF		NM_003131	964	Beta-scaffold-MADS	serum response factor (c-fos serum response element-binding transcription factor)
SRY		NM_003140	965	Beta-scaffold-HMG	sex determining region Y
SSA1	NT_028310:75	NM_003141	966	Structural	Sjogren syndrome antigen A1 (52 kDa, ribonucleoprotein autoantigen SS-A/Ro)
SSRP1		NM_003146	967	Co-activator	structure specific recognition protein 1
SSX1		NM_005635	968	Other	synovial sarcoma, X breakpoint 1
SSX2		NM_003147	969	Other	synovial sarcoma, X breakpoint 2

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
SSX3		NM_021014	970	Other	synovial sarcoma, X breakpoint 3
SSX4		NM_005636	971	Other	synovial sarcoma, X breakpoint 4
SSX5		NM_021015	972	Other	synovial sarcoma, X breakpoint 5
SSX6		NM_173357	973	Other	synovial sarcoma, X breakpoint 6
SSX7		NM_173358	974	Other	synovial sarcoma, X breakpoint 7
SSX8		NM_174961	975	Other	synovial sarcoma, X breakpoint 8
SSX9		NM_174962	976	Other	synovial sarcoma, X breakpoint 9
ST18		NM_014682	977	ZnF-C3H	suppression of tumorigenicity 18 (breast carcinoma) (zinc finger protein)
STAT1		NM_007315	978	Beta-scaffold-STAT	signal transducer and activator of transcription 1, 91kDa
STAT2		NM_005419	979	Beta-scaffold-STAT	signal transducer and activator of transcription 2, 113kDa
STAT3		NM_003150	980	Beta-scaffold-STAT	signal transducer and activator of transcription 3 (acute-phase response factor)
STAT4		NM_003151	981	Beta-scaffold-STAT	signal transducer and activator of transcription 4
STAT5A		NM_003152	982	Beta-scaffold-STAT	signal transducer and activator of transcription 5A
STAT5B		NM_012448	983	Beta-scaffold-STAT	signal transducer and activator of transcription 5B
STAT6		NM_003153	984	Beta-scaffold-STAT	signal transducer and activator of transcription 6, interleukin-4 induced
SUPT16H		NM_007192	985	Other	suppressor of Ty 16 homolog (S. cerevisiae)
SUPT3H		NM_003599	986	Other	suppressor of Ty 3 homolog (S.cerevisiae)
SUPT4H1		NM_003168	987	Other	suppressor of Ty 4 homolog (S.cerevisiae)
SUPT5H		NM_003169	988	Dwarfin	suppressor of Ty 5 homolog (S.cerevisiae)
SUPT6H		NM_003170	989	Other	suppressor of Ty 6 homolog (S.cerevisiae)
SURB7		NM_004264	990	Other	SRB7 suppressor of RNA polymerase B homolog (yeast)
SUV39H1	NT_011568:277	NM_003173	991	Structural	suppressor of variegation 3-9 homolog 1 (Drosophila)
SZF1:	NT_022567:166	NM_016089	992	ZnF-C2H2	KRAB-zinc finger protein

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					SZF1-1
SZFP41	NT_011192:184	NM_152279	993	ZnF-C2H2	zinc finger protein 41-like
T		NM_003181	994	T-box	T, brachyury homolog (mouse)
TADA2L		NM_001488	995	Other	transcriptional adaptor 2 (ADA2 homolog, yeast)-like
TADA3L		NM_006354	996	Other	transcriptional adaptor 3 (ADA3 homolog, yeast)-like
TAF1		NM_004606	997	Other	TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 250kDa
TAF10		NM_006284	998	Other	TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 30kDa
TAF11		NM_005643	999	Other	TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 28kDa
TAF12		NM_005644	1000	Other	TAF12 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 20kDa
TAF13		NM_005645	1001	Other	TAF13 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 18kDa
TAF15		NM_003487	1002	Other	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68kDa
TAF1A		NM_005681	1003	Other	TATA box binding protein (TBP)-associated factor, RNA polymerase I, A, 48kDa
TAF1B		L39061	1004	Other	TATA box binding protein (TBP)-associated factor, RNA polymerase 1, B, 63kDa
TAF1C		NM_005679	1005	Other	TATA box binding protein (TBP)-associated factor, RNA polymerase I, C, 110kDa
TAF2		NM_003184	1006	Other	TAF2 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 150kDa
TAF3		AJ292190	1007	Other	TAF3 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 140kDa
TAF4		NM_003185	1008	Other	TAF4 RNA polymerase II, TATA box binding protein

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					(TBP)-associated factor, 135kDa
TAF4B		Y09321	1009	Other	TAF4b RNA polymerase II, TATA box binding protein (TBP)-associated factor, 80kDa
TAF6L		NM_006473	1010	Co-activator	TAF6-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor, 65kDa
TAF7		NM_005642	1011	Other	TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 55kDa
TAF9		NM_003187	1012	Other	TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 32kDa
TAL1		NM_003189	1013	bHLH	T-cell acute lymphocytic leukemia 1
TAL2		NM_005421	1014	bHLH	T-cell acute lymphocytic leukemia 2
TBP		NM_003194	1015	Other	TATA box binding protein
TBPL1		NM_004865	1016	Other	TBP-like 1
TBR1		NM_006593	1017	T-box	T-box, brain, 1
TBX1		NM_005992	1018	T-box	T-box 1
TBX10		AF033579	1019	T-box	T-box 10
TBX15		NM_152380	1020	T-box	T-box 15
TBX18		AJ010278	1021	T-box	T-box 18
TBX19		NM_005149	1022	T-box	T-box 19
TBX2		NM_005994	1023	T-box	T-box 2
TBX20		AJ237589	1024	T-box	T-box 20
TBX21		NM_013351	1025	T-box	T-box 21
TBX22		NM_016954	1026	T-box	T-box 22
TBX3		NM_005996	1027	T-box	T-box 3 (ulnar mammary syndrome)
TBX4		NM_018488	1028	T-box	T-box 4
TBX5		NM_000192	1029	T-box	T-box 5
TBX6		NM_004608	1030	T-box	T-box 6
TCEAL1		NM_004780	1031	ZnF-Other	transcription elongation factor A (SII)-like 1
TCERG1		NM_006706	1032	Other	transcription elongation regulator 1 (CA150)
TCF1		NM_000545	1033	Homeobox	transcription factor 1, hepatic; LF-B1, hepatic nuclear factor (HNF1), albumin proximal factor
TCF12		NM_003205	1034	bHLH	transcription factor 12 (HTF4, helix-loop-helix transcription factors 4)
TCF15		NM_004609	1035	bHLH	transcription factor 15 (basic helix-loop-helix)
TCF19		NM_007109	1036	Other	transcription factor 19 (SC1)
TCF7		NM_003202	1037	scaffold-HMG	transcription factor 2, (T-cell specific, HMG-box)

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TCF7L1		X62870	1038	Beta-scaffold-HMG	transcription factor 7-like 1 (T-cell specific, HMG-box)
TCF7L2		NM_030756	1039	Beta-scaffold-HMG	transcription factor 7-like 2 (T-cell specific, HMG-box)
TCF8		NM_030751	1040	ZnF-C2H2	transcription factor 8 (represses interleukin 2 expression)
TCFL1		NM_005997	1041	Other	transcription factor-like 1
TCFL4		NM_013383	1042	bHLH	transcription factor-like 4
TCFL5		NM_006602	1043	bHLH	transcription factor-like 5 (basic helix-loop-helix)
TEAD1		NM_021961	1044	TEA	TEA domain family member 1 (SV40 transcriptional enhancer factor)
TEAD2		NM_003598	1045	TEA	TEA domain family member 2
TEAD3		NM_003214	1046	TEA	TEA domain family member 3
TEAD4		NM_003213	1047	TEA	TEA domain family member 4
TEF		NM_003216	1048	bZIP	thyrotrophic embryonic factor
TEL2		NM_016135	1049	Trp cluster-Ets	ets transcription factor TEL2
TEX27		NM_021943	1050	ZnF-AN1	testis expressed sequence 27
TFAM		NM_012251	1051	Beta-scaffold-HMG	transcription factor A, mitochondrial
TFAP2A		NM_003220	1052	AP-2	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)
TFAP2B		NM_003221	1053	AP-2	transcription factor AP-2 beta (activating enhancer binding protein 2 beta)
TFAP2BL1		NM_172238	1054	AP-2	transcription factor AP-2 beta (activating enhancer binding protein 2 beta)-like 1
TFAP2C		NM_003222	1055	AP-2	transcription factor AP-2 gamma (activating enhancer binding protein 2 gamma)
TFAP4		NM_003223	1056	bHLH	transcription factor AP-4 (activating enhancer binding protein 4)
TFB1M		NM_016020	1057	Other	transcription factor B1, mitochondrial
TFB2M		NM_022366	1058	Other	transcription factor B2, mitochondrial
TFCP2		NM_005653	1059	Beta-scaffold-grainyhead	transcription factor CP2
TFE3		NM_006521	1060	bHLH	transcription factor binding to IGHM

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					enhancer 3
TFEB		BC006225	1061	bHLH	transcription factor EB
TFEC		NM_012252	1062	bHLH	transcription factor EC
TGFB1I1		NM_015927	1063	Co-activator	transforming growth factor beta 1 induced transcript 1
TGIF		NM_003244	1064	Homeobox	TGFB-induced factor (TALE family homeobox)
THG-1		AJ133115	1065	bZIP	TSC-22-like
THRA		NM_003250	1066	NHR	thyroid hormone receptor, alpha (erythroblastic leukemia viral (v-erb-a) oncogene homolog, avian)
THRAP4		NM_014815	1067	Co-activator	thyroid hormone receptor associated protein 4
THRB		NM_000461	1068	NHR	thyroid hormone receptor, beta (erythroblastic leukemia viral (v-erb-a) oncogene homolog 2, avian)
TIEG		NM_005655	1069	ZnF-C2H2	TGFB inducible early growth response
TIEG2		NM_003597	1070	ZnF-C2H2	TGFB inducible early growth response 2
TIF1		NM_003852	1071	Structural	transcriptional intermediary factor 1
TIMELESS		NM_003920	1072	Other	timeless homolog (Drosophila)
TIP120A		NM_018448	1073	Co-activator	TBP-interacting protein
TITF1		NM_003317	1074	Homeobox	thyroid transcription factor 1
TIX1		AB007855	1075	Homeobox	triple homeobox 1
TIZ	NT_033317:106	NM_138330	1076	ZnF-C2H2	TRAF6-inhibitory zinc finger protein
TLX1		NM_005521	1077	Homeobox	T-cell leukemia, homeobox 1
TLX2		NM_001534	1078	Homeobox	T-cell leukemia, homeobox 2
TLX3		NM_021025	1079	Homeobox	T-cell leukemia, homeobox 3
TMF1		NM_007114	1080	Other	TATA element modulatory factor 1
TNRC11		NM_005120	1081	Co-activator	trinucleotide repeat containing 11 (THR-associated protein, 230 kDa subunit)
TNRC17		U80752.1	1082	Other	trinucleotide repeat containing 17
TNRC18		U80753	1083	Other	trinucleotide repeat containing 18
TNRC21		U80756	1084	Other	trinucleotide repeat containing 21
TNRC3		NM_005878	1085	Other	trinucleotide repeat containing 3
TP53		NM_000546	1086	Beta-scaffold-p53	tumor protein P53 (Li-Fraumeni syndrome)
TP53BP2	NT_004525:42	NM_005426	1087	Co-repressor	tumor protein p53 binding

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					protein, 2
TP63		NM_003722	1088	Beta-scaffold-p53	tumor protein p63
TP73		NM_005427	1089	Beta-scaffold-p53	tumor protein p73
TRAP150		NM_005119	1090	Co-activator	thyroid hormone receptor-associated protein, 150 kDa subunit
TRAP95		NM_005481	1091	Co-activator	thyroid hormone receptor-associated protein, 95-kD subunit
TRERF1	NT_007592:3400	NM_018415	1092	ZnF-C2H2	transcriptional regulating factor 1
TRIM10		NM_006778	1093	Structural	tripartite motif-containing 10
TRIM14	NT_033216:170	NM_014788	1094	Structural	tripartite motif-containing 14
TRIM15		NM_033229	1095	Structural	tripartite motif-containing 15
TRIM16	NT_010718:517	NM_006470	1096	Structural	tripartite motif-containing 16
TRIM17	NT_004908:93	NM_016102	1097	Structural	tripartite motif-containing 17
TRIM22		NM_006074	1098	Structural	tripartite motif-containing 22
TRIM26		NM_003449	1099	Structural	tripartite motif-containing 26
TRIM28		NM_005762	1100	Structural	tripartite motif-containing 28
TRIM29	NT_033899:65	NM_012101	1101	Structural	tripartite motif-containing 29
TRIM3		NM_006458	1102	ZnF-Other	tripartite motif-containing 3
TRIM31	NT_034873:26	NM_007028	1103	Structural	tripartite motif-containing 31
TRIM33		NM_015906	1104	Structural	tripartite motif-containing 33
TRIM34	NT_03508:27a	NM_021616	1105	Structural	tripartite motif-containing 34
TRIM35	NT_007988:5	NM_015066	1106	Structural	tripartite motif-containing 35
TRIM38		NM_006355	1107	ZnF-Other	tripartite motif-containing 38
TRIM39	NT_033951:12	NM_021253	1108	Structural	tripartite motif-containing 39
TRIM4	NT_007933:2024	NM_033017	1109	Structural	tripartite motif-containing 4
TRIM40	NT_007592:1918	NM_138700	1110	Structural	tripartite motif-containing 40
TRIM41	NT_006519:206	NM_201627	1111	Structural	tripartite motif-containing 41
TRIM47	NT_033292:11	NM_033452	1112	Structural	tripartite motif-containing 47
TRIM48	NT_033903:1	NM_024114	1113	Structural	tripartite motif-containing 48
TRIM5	NT_035080:27b	NM_033034	1114	Structural	tripartite motif-containing

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					5
TRIP11		NM_004239	1115	Co-activator	thyroid hormone receptor interactor 11
TRIP11		NM_004237	1116	Co-activator	thyroid hormone receptor interactor 13
TRIP15		NM_004236	1117	Co-activator	thyroid receptor interacting protein 15
TRIP4		NM_016213	1118	Co-activator	thyroid hormone receptor interactor 4
TRIP6		L40374	1119	Co-activator	thyroid hormone receptor interactor 6
TRIP8	NT_008583:38	NM_004241	1120	Jumonji	thyroid hormone receptor interactor 8
TRIP-Br2		NM_014755	1121	Co-activator	transcriptional regulator interacting with the PHS-bromodomain 2
TRPS1		NM_014112	1122	ZnF-Other	trichorhinophalangeal syndrome I
TSC22		NM_006022	1123	bZIP	transforming growth factor beta-stimulated protein TSC-22
TUB		NM_003320	1124	Tubby	tubby homolog (mouse)
TULP1		NM_003322	1125	Tubby	tubby like protein 1
TULP2		NM_003323	1126	Tubby	tubby like protein 2
TULP3		NM_003324	1127	Tubby	tubby like protein 3
TULP4		NM_020245	1128	Tubby	tubby like protein 4
TWIST		NM_000474	1129	bHLH	Twist
TZFP		NM_014383	1130	ZnF-BTB/POZ	testis zinc finger protein
UBP1		NM_014517	1131	Beta-scaffold-grainyhead	upstream binding protein 1 (LBP-1a)
UBTF		NM_014233	1132	Beta-scaffold-HMG	upstream binding transcription factor, RNA polymerase 1
UHRF1		NM_013282	1133	ZnF-PHD	ubiquitin-like, containing PHD and RING finger
URF2	NT_008413:704	NM_152306	1134	ZnF-PHD	ubiquitin-like, containing PHD and RING finger domains 2
USF1		NM_007122	1135	bHLH	upstream transcription factor 1
USF2		NM_003367	1136	bHLH	upstream transcription factor 2, c-fos interacting
UTF1		NM_003577	1137	bZIP	undifferentiated embryonic cell transcription factor 1
VAX1		NM_199131	1138	Homeobox	ventral anterior homeobox 1
VAX2		NM_012476	1139	Homeobox	ventral anterior homeobox 2
VDR		NM_000376	1140	NHR	vitamin D (1,25-dihydroxyvitamin D3) receptor
VENTX2		NM_014468	1141	Homeobox	VENT-like homeobox 2
VIK	NT_007933:1990	NM_024061	1142	ZnF-C2H2	vav-1 interacting Kruppel-like protein

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
cutoff					
YAF2		NM_005748	1143	Co-repressor	YY1 associated factor 2
YBX2		NM_015982	1144	Beta-scaffold-cold-shock	germ cell specific Y-box binding protein
YY1		NM_003403	1145	ZnF-C2H2	YY1 transcription factor
ZAR1		NM_175619	1146	Other	zygote arrest 1
ZBTB1	NT_025892:3338	BC050719	1147	ZnF-BTB/POZ	zinc finger and BTB domain containing 1
ZBTB2	NT_023451:235	NM_020861	1148	ZnF-BTB/POZ	zinc finger and BTB domain containing 2
ZBTB4	NT_035416:6	NM_020899	1149	ZnF-C2H2	zinc finger and BTB domain containing 4
ZDHHC1		U90653	1150	ZnF-Other	zinc finger, DHHC domain containing 1
ZF		NM_021212	1151	bZIP	HCF-binding transcription factor Zhangfei
ZF5128		NM_014347	1152	ZnF-C2H2	zinc finger protein
ZFD25		NM_016220	1153	ZnF-C2H2	zinc finger protein (ZFD25)
ZFH4	NT_008055:104	NM_024721	1154	ZnF-C2H2	zinc finger homeodomain 4
ZFHX1B		NM_014795	1155	ZnF-C2H2	zinc finger homeobox 1B
ZFHX2		AB051549	1156	Homeobox	zinc finger homeobox 2
ZFP		NM_018651	1157	ZnF-C2H2	zinc finger protein
ZFP1	NT_035368:196	NM_153688	1158	ZnF-C2H2	zinc finger protein homolog
ZFP100		AL080143	1159	ZnF-C2H2	zinc finger protein
ZFP103		NM_005677	1160	ZnF-Other	zinc finger protein 103 homolog (mouse)
ZFP106		NM_022473	1161	ZnF-C2H2	zinc finger protein 106
ZFP161		NM_003409	1162	ZnF-BTB/POZ	zinc finger protein 161 homolog (mouse)
ZFP26		NM_016422	1163	ZnF-Other	C3HC4-like zinc finger protein
ZFP276	NT_010542:164	NM_152287	1164	ZnF-C2H2	zinc finger protein 276 homolog
ZFP28		AB037852	1165	ZnF-C2H2	zinc finger protein 28 homolog (mouse)
ZFP289		NM_032389	1166	ZnF-Other	Seed zinc finger protein 289, ID1 regulated
ZFP29		NM_017894	1167	ZnF-C2H2	likely ortholog of mouse zinc finger protein 29
ZFP318		NM_014345	1168	ZnF-Other	Seed endocrine regulator
ZFP36		NM_003407	1169	ZnF-C3H	zinc finger protein 36, C3H type, homolog (mouse)
ZFP37		NM_003408	1170	ZnF-C2H2	zinc finger protein 37 homolog (mouse)
ZFP42	NT_022841:73	NM_174900	1171	ZnF-C2H2	Found zinc finger protein 42
ZFP64		NM_018197	1172	ZnF-C2H2	Seed zinc finger protein 64 homolog (mouse)
ZFP67		NM_015872	1173	ZnF-BTB/POZ	Seed zinc finger protein 67 homolog (mouse)
ZFP91		AB056107	1174	ZnF-C2H2	zinc finger protein 91 homolog (mouse)

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ZFP92		U82695	1175	ZnF-Other	zinc finger protein 92 homolog (mouse)
ZFP95		NM_014569	1176	ZnF-C2H2	zinc finger protein 95 homolog (mouse)
ZFPL1		NM_006782	1177	ZnF-PHD	zinc finger protein-like 1
ZFPM1		NM_153813	1178	ZnF-C2H2	zinc finger protein, multitype 1 (FOG1)
ZFPM2		NM_012082	1179	ZnF-C2H2	zinc finger protein, multitype 2 (FOG2)
ZFR		NM_016107	1180	ZnF-C2H2	zinc finger RNA binding protein
ZFX		NM_003410	1181	ZnF-C2H2	zinc finger protein, X-linked
ZFY		NM_003411	1182	ZnF-C2H2	zinc finger protein, Y-linked
ZHX1		NM_007222	1183	Homeobox	zinc-fingers and homeoboxes 1
ZHX2	NT_023663:37	NM_014943	1184	Homeobox	zinc fingers and homeoboxes 2
ZIC1		NM_003412	1185	ZnF-C2H2	Zic family member 1 (odd-paired homolog, Drosophila)
ZIC2		NM_007129	1186	ZnF-C2H2	Zic family member 2 (odd-paired homolog, Drosophila)
ZIC3		NM_003413	1187	ZnF-C2H2	Zic family member 3 heterotaxy 1 (odd-paired homolog, Drosophila)
ZIC4		NM_032153	1188	ZnF-C2H2	zinc finger protein of the cerebellum 4
ZIC5		NM_033132	1189	ZnF-C2H2	zinc finger protein of the cerebellum 5
ZID		NM_006626	1190	ZnF-BTB/POZ	zinc finger protein with interaction domain
ZIM2		NM_015363	1191	ZnF-C2H2	zinc finger, imprinted 2
ZIM3	NT_011104:125	NM_052882	1192	ZnF-C2H2	zinc finger, imprinted 3
ZNF10		NM_003419	1193	ZnF-C2H2	zinc finger protein 10 (KOX 1)
ZNF100	NT_035560:167	NM_173531	1194	ZnF-C2H2	zinc finger protein 100
ZNF117		NM_024498	1195	ZnF-C2H2	zinc finger protein 117 (HPF9)
ZNF11A		X68686	1196	ZnF-C2H2	zinc finger protein 11a (KOX 2)
ZNF11B		X68684	1197	ZnF-C2H2	zinc finger protein 11b (KOX 2)
ZNF123		S52506	1198	ZnF-C2H2	zinc finger protein 123 (HZF-1)
ZNF124		NM_003431	1199	ZnF-C2H2	zinc finger protein 124 (HZF-16)
ZNF125		S52508	1200	ZnF-C2H2	zinc finger protein 125 (HZF-3)
ZNF126		S52507	1201	ZnF-C2H2	zinc finger protein 126 (HZF-2)
ZNF131		U09410	1202	ZnF-C2H2	zinc finger protein 131 (clone pHZ-10)
ZNF132		NM_003433	1203	ZnF-C2H2	zinc finger protein 132

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
					(clone pHZ-12)
ZNF133		NM_003434	1204	ZnF-C2H2	zinc finger protein 133 (clone pHZ-13)
ZNF134		NM_003435	1205	ZnF-C2H2	zinc finger protein 134 (clone pHZ-15)
ZNF135		NM_003436	1206	ZnF-C2H2	zinc finger protein 135 (clone pHZ-17)
ZNF136		NM_003437	1207	ZnF-C2H2	zinc finger protein 136 (clone pHZ-20)
ZNF137		NM_003438	1208	ZnF-C2H2	zinc finger protein 137 (clone pHZ-30)
ZNF138		U09847	1209	ZnF-C2H2	zinc finger protein 138 (clone pHZ-32)
ZNF14		NM_021030	1210	ZnF-C2H2	zinc finger protein 14 (KOX 6)
ZNF140		NM_003440	1211	ZnF-C2H2	zinc finger protein 140 (clone pHZ-39)
ZNF141		NM_003441	1212	ZnF-C2H2	zinc finger protein 141 (clone pHZ-44)
ZNF142		NM_005081	1213	ZnF-C2H2	zinc finger protein 142 (clone pHZ-49)
ZNF143		NM_003442	1214	ZnF-C2H2	zinc finger protein 143 (clone pHZ-1)
ZNF144		NM_007144	1215	ZnF-Other	zinc finger protein 144 (Mel-18)
ZNF145		NM_006006	1216	ZnF-BTB/POZ	zinc finger protein 145 (Kruppel-like, expressed in promyelocytic leukemia)
ZNF146		NM_007145	1217	ZnF-C2H2	zinc finger protein 146
ZNF147		NM_005082	1218	Structural	zinc finger protein 147 (estrogen-responsive finger protein)
ZNF148		NM_021964	1219	ZnF-C2H2	zinc finger protein 148 (pHZ-52)
ZNF151		NM_003443	1220	ZnF-BTB/POZ	zinc finger protein 151 (pHZ-67)
ZNF154		U20648	1221	ZnF-C2H2	zinc finger protein 154 (pHZ-92)
ZNF155		NM_003445	1222	ZnF-C2H2	zinc finger protein 155 (pHZ-96)
ZNF157		NM_003446	1223	ZnF-C2H2	zinc finger protein 157 (HZF22)
ZNF15L1		X52339	1224	ZnF-C2H2	zinc finger protein 15-like 1 (KOX 8)
ZNF16		NM_006958	1225	ZnF-C2H2	zinc finger protein 16 (KOX 9)
ZNF160		X78928	1226	ZnF-C2H2	zinc finger protein 160
ZNF161		NM_007146	1227	ZnF-C2H2	zinc finger protein 161
ZNF165		NM_003447	1228	ZnF-C2H2	zinc finger protein 165
ZNF169		U28251	1229	ZnF-C2H2	zinc finger protein 169
ZNF17		AB075827	1230	ZnF-C2H2	zinc finger protein 17 (HPF3, KOX 10)
ZNF174		NM_003450	1231	ZnF-C2H2	zinc finger protein 174
ZNF175		NM_007147	1232	ZnF-C2H2	zinc finger protein 175
ZNF177		NM_003451	1233	ZnF-C2H2	zinc finger protein 177

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
ZNF179		NM_007148	1234	ZnF-Other	zinc finger protein 179
ZNF18		X52342	1235	ZnF-C2H2	zinc finger protein 18 (KOX 11)
ZNF180		NM_013256	1236	ZnF-C2H2	zinc finger protein 180 (HHZ168)
ZNF183		NM_006978	1237	ZnF-Other	zinc finger protein 183 (RING finger, C3HC4 type)
ZNF183L1	NT_009952:601	NM_178861	1238	ZnF-C3H	zinc finger protein 183-like 1
ZNF184		U66561	1239	ZnF-C2H2	zinc finger protein 184 (Kruppel-like)
ZNF185		NM_007150	1240	Co-activator	zinc finger protein 185 (LIM domain)
ZNF187		Z11773	1241	ZnF-C2H2	zinc finger protein 187
ZNF189		NM_003452	1242	ZnF-C2H2	zinc finger protein 189
ZNF19		NM_006961	1243	ZnF-C2H2	zinc finger protein 19 (KOX 12)
ZNF192		NM_006298	1244	ZnF-C2H2	zinc finger protein 192
ZNF193		NM_006299	1245	ZnF-C2H2	zinc finger protein 193
ZNF195		NM_007152	1246	ZnF-C2H2	zinc finger protein 195
ZNF197		NM_006991	1247	ZnF-C2H2	zinc finger protein 197
ZNF2		Z60152	1248	ZnF-C2H2	zinc finger protein 2 (A1-5)
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ZNF202		NM_003455	1251	ZnF-C2H2	zinc finger protein 202
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ZNF207		NM_003457	1253	ZnF-C2H2	zinc finger protein 207
ZNF208		NM_007153	1254	ZnF-C2H2	zinc finger protein 208
ZNF21		X52345	1255	ZnF-C2H2	zinc finger protein 21 (KOX 14)
ZNF211		NM_006385	1256	ZnF-C2H2	zinc finger protein 211
ZNF212		NM_012256	1257	ZnF-C2H2	zinc finger protein 212
ZNF213		AF017433	1258	ZnF-C2H2	zinc finger protein 213
ZNF214		NM_013249	1259	ZnF-C2H2	zinc finger protein 214
ZNF215		NM_013250	1260	ZnF-C2H2	zinc finger protein 215
ZNF216		NM_006007	1261	ZnF-AN1	zinc finger protein 216
ZNF217		NM_006526	1262	ZnF-C2H2	zinc finger protein 217
ZNF219		NM_016423	1263	ZnF-C2H2	zinc finger protein 219
ZNF22		NM_006963	1264	ZnF-C2H2	zinc finger protein 22 (KOX 15)
ZNF220		NM_006766	1265	ZnF-PHD	zinc finger protein 220
ZNF221		NM_013359	1266	ZnF-C2H2	zinc finger protein 221
ZNF222		NM_013360	1267	ZnF-C2H2	zinc finger protein 222
ZNF223		NM_013361	1268	ZnF-C2H2	zinc finger protein 223
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ZNF225		NM_013362	1270	ZnF-C2H2	zinc finger protein 225
ZNF226		NM_016444	1271	ZnF-C2H2	zinc finger protein 226
ZNF228		NM_013380	1272	ZnF-C2H2	zinc finger protein 228
ZNF229		AF192979	1273	ZnF-C2H2	zinc finger protein 229
ZNF23		AL080123	1274	ZnF-C2H2	zinc finger protein 23 (KOX 16)
ZNF230		NM_006300	1275	ZnF-C2H2	zinc finger protein 230

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
ZNF232		NM_014519	1276	ZnF-C2H2	zinc finger protein 232
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ZNF235		NM_004234	1279	ZnF-C2H2	zinc finger protein 235
ZNF236		NM_007345	1280	ZnF-C2H2	zinc finger protein 236
ZNF237		NM_014242	1281	ZnF-Other	zinc finger protein 237
ZNF238		NM_006352	1282	ZnF-C2H2	zinc finger protein 238
ZNF239		NM_005674	1283	ZnF-C2H2	zinc finger protein 239
ZNF24		NM_006965	1284	ZnF-C2H2	zinc finger protein 24 (KOX 17)
ZNF25		X52350	1285	ZnF-C2H2	zinc finger protein 25 (KOX 19)
ZNF253	NT_011295:613	NM_021047	1286	ZnF-C2H2	zinc finger protein 253
ZNF254		NM_004876	1287	ZnF-C2H2	zinc finger protein 254
ZNF255		NM_005774	1288	ZnF-C2H2	zinc finger protein 255
ZNF256		NM_005773	1289	ZnF-C2H2	zinc finger protein 256
ZNF257	NT_033317:9	NM_033468	1290	ZnF-C2H2	zinc finger protein 257
ZNF258		NM_007167	1291	ZnF-Other	zinc finger protein 258
ZNF259		NM_003904	1292	ZnF-Other	zinc finger protein 259
ZNF26		NM_019591	1293	ZnF-C2H2	zinc finger protein 26 (KOX 20)
ZNF261		NM_005096	1294	ZnF-Other	zinc finger protein 261
ZNF262		NM_005095	1295	ZnF-Other	zinc finger protein 262
ZNF263		NM_005741	1296	ZnF-C2H2	zinc finger protein 263
ZNF264		NM_003417	1297	ZnF-C2H2	zinc finger protein 264
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ZNF273		X78932	1304	ZnF-C2H2	zinc finger protein 273
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ZNF275		NM_020636	1306	ZnF-C2H2	zinc finger protein 275
ZNF277		NM_021994	1307	ZnF-C2H2	zinc finger protein (C2H2 type) 277
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ZNF281		NM_012482	1309	ZnF-C2H2	zinc finger protein 281
ZNF282		D30612	1310	ZnF-C2H2	zinc finger protein 282
ZNF286		NM_020652	1311	ZnF-C2H2	zinc finger protein 286
ZNF287		NM_020653	1312	ZnF-C2H2	zinc finger protein 287
ZNF288		NM_015642	1313	ZnF-BTB/POZ	zinc finger protein 288
ZNF29		X52357	1314	ZnF-C2H2	zinc finger protein 29 (KOX 26)
ZNF294		AB018257	1315	ZnF-Other	zinc finger protein 294
ZNF295		NM_020727	1316	ZnF-BTB/POZ	zinc finger protein 295
ZNF297		NM_005453	1317	ZnF-BTB/POZ	zinc finger protein 297
ZNF297B		NM_014007	1318	ZnF-BTB/POZ	zinc finger protein 297B
ZNF3		NM_017715	1319	ZnF-C2H2	zinc finger protein 3 (A8-51)

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
ZNF30		X52359	1320	ZnF-C2H2	zinc finger protein 30 (KOX 28)
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ZNF302	NT_011196:498	NM_018443	1322	ZnF-C2H2	zinc finger protein 302
ZNF304		NM_020657	1323	ZnF-C2H2	zinc finger protein 304
ZNF305		NM_014724	1324	ZnF-C2H2	zinc finger protein 305
ZNF306		NM_024493	1325	ZnF-C2H2	zinc finger protein 306
ZNF31		NM_145238	1326	ZnF-C2H2	zinc finger protein 31 (KOX 29)
ZNF313		NM_018683	1327	ZnF-Other	zinc finger protein 313
ZNF317	NT_011176:75	NM_020933	1328	ZnF-C2H2	zinc finger protein 317
ZNF319		AB037809	1329	ZnF-C2H2	zinc finger protein 319
ZNF32		NM_006973	1330	ZnF-C2H2	zinc finger protein 32 (KOX 30)
ZNF322A	NT_007592:1565	NM_024639	1331	ZnF-PHD	zinc finger protein 322A
ZNF323	NT_007592:1771	NM_030899	1332	ZnF-C2H2	zinc finger protein 323
ZNF325		NM_016265	1333	ZnF-C2H2	zinc finger protein 325
ZNF333	NT_025155:3	NM_032433	1334	ZnF-C2H2	zinc finger protein 333
ZNF334		NM_018102	1335	ZnF-C2H2	zinc finger protein 334
ZNF335	NT_011362:859	NM_022095	1336	ZnF-C2H2	zinc finger protein 335
ZNF336	NT_011387:1856	NM_022482	1337	ZnF-C2H2	zinc finger protein 336
ZNF337		AL049942	1338	ZnF-C2H2	zinc finger protein 337
ZNF339	NT_011387:1400	NM_021220	1339	ZnF-C2H2	zinc finger protein 339
ZNF33A		X68687	1340	ZnF-C2H2	zinc finger protein 33a (KOX 31)
ZNF341	NT_028392:330	NM_032819	1341	ZnF-C2H2	zinc finger protein 341
ZNF342	NT_011109:256	NM_145288	1342	ZnF-C2H2	zinc finger protein 342
ZNF347	NT_011109:1491	NM_032584	1343	ZnF-C2H2	zinc finger protein 347
ZNF35		NM_003420	1344	ZnF-C2H2	zinc finger protein 35 (clone HF.10)
ZNF350	NT_011109:1276	NM_021632	1345	ZnF-C2H2	zinc finger protein 350
ZNF354A		NM_005649	1346	ZnF-C2H2	zinc finger protein 354A
ZNF358		NM_018083	1347	ZnF-C2H2	zinc finger protein 358
ZNF36		U09848	1348	ZnF-C2H2	zinc finger protein 36 (KOX 18)
ZNF361		NM_018555	1349	ZnF-C2H2	zinc finger protein 361
ZNF364		AL079314	1350	ZnF-Other	zinc finger protein 364
ZNF366	NT_006713:99	NM_152625	1351	ZnF-C2H2	zinc finger protein 366
ZNF37A		X69115	1352	ZnF-C2H2	zinc finger protein 37a (KOX 21)
ZNF37A	NT_033896:447	AJ492195	1353	ZnF-C2H2	zinc finger protein 37a (KOX21)
ZNF38		NM_032924	1354	ZnF-C2H2	zinc finger protein 38
ZNF382	NT_011192:90	NM_032825	1355	ZnF-C2H2	zinc finger protein ZNF382
ZNF384	NT_009731:144	NM_133476	1356	ZnF-C2H2	zinc finger protein 384
ZNF394	NT_007933:1972	NM_032164	1357	ZnF-C2H2	zinc finger protein 394
ZNF396	NT_010934:143	NM_145756	1358	ZnF-C2H2	zinc finger protein 396
ZNF397	NT_010934:119	NM_032347	1359	ZnF-C2H2	zinc finger protein 397
ZNF398	NT_007914:756	NM_020781	1360	ZnF-C2H2	zinc finger protein 398
ZNF406	NT_007994:1	AB040918	1361	ZnF-C2H2	zinc finger protein 406
ZNF407	NT_025004:1	NM_017757	1362	ZnF-C2H2	zinc finger protein 407
ZNF408		NM_024741	1363	ZnF-C2H2	zinc finger protein 408
ZNF409	NT_025892:468	AB028979	1364	ZnF-C2H2	zinc finger protein 409
ZNF41		M92443	1365	ZnF-C2H2	zinc finger protein 41

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
ZNF42		NM_003422	1366	ZnF-C2H2	zinc finger protein 42 (myeloid-specific retinoic acid-responsive)
ZNF426	NT_011176:123	NM_024106	1367	ZnF-C2H2	zinc finger protein 426
ZNF43		NM_003423	1368	ZnF-C2H2	zinc finger protein 43 (HTF6)
ZNF431	NT_035560:82	NM_133473	1369	ZnF-C2H2	zinc finger protein 431
ZNF433	NT_011176:487	NM_152602	1370	ZnF-C2H2	zinc finger protein 433
ZNF434	NT_010552:596	NM_017810	1371	ZnF-C2H2	zinc finger protein 434
ZNF435	NT_007592:1726	NM_025231	1372	ZnF-C2H2	zinc finger protein 435
ZNF436	NT_032979:37	NM_030634	1373	ZnF-C2H2	zinc finger protein 436
ZNF44		X16281	1374	ZnF-C2H2	zinc finger protein 44 (KOX 7)
ZNF440	NT_011176:446	NM_152357	1375	ZnF-AN1	zinc finger protein 440
ZNF443		NM_005815	1376	ZnF-C2H2	zinc finger protein 443
ZNF445	NT_034534:46	NM_181489	1377	ZnF-C2H2	zinc finger protein 445
ZNF45		NM_003425	1378	ZnF-C2H2	zinc finger protein 45 (a Kruppel-associated box (KRAB) domain polypeptide)
ZNF454	NT_006802:20	NM_182594	1379	ZnF-C2H2	zinc finger protein 454
ZNF46		NM_006977	1380	ZnF-BTB/POZ	zinc finger protein 46 (KUP)
ZNF481	NT_017568:1387	NM_020924	1381	ZnF-BTB/POZ	zinc finger protein 481
ZNF486	NT_035560:14	BC008936	1382	ZnF-C2H2	zinc finger protein 486
ZNF490	NT_011176:576	NM_020714	1383	ZnF-C2H2	zinc finger protein 490
ZNF491	NT_011176:438	NM_152356	1384	ZnF-C2H2	zinc finger protein 491
ZNF493	NT_035560:126b	NM_175910	1385	ZnF-C2H2	zinc finger protein 493
ZNF494	NT_011104:214	NM_152677	1386	ZnF-C2H2	zinc finger protein 494
ZNF495	NT_011104:32a	NM_024303	1387	ZnF-C2H2	zinc finger protein 495
ZNF496	NT_031730:64	NM_032752	1388	ZnF-C2H2	zinc finger protein 496
ZNF497	NT_011104:359	NM_198458	1389	ZnF-C2H2	zinc finger protein 497
ZNF498	NT_007933:1998	NM_145115	1390	ZnF-C2H2	zinc finger protein 498
ZNF502	NT_034534:1	NM_033210	1391	ZnF-C2H2	zinc finger protein 502
ZNF503	NT_033890:224	NM_032772	1392	ZnF-C2H2	zinc finger protein 503
ZNF509	NT_006051:22	NM_145291	1393	ZnF-BTB/POZ	zinc finger protein 509
ZNF513	NT_005204:559	NM_144631	1394	ZnF-C2H2	zinc finger protein 513
ZNF514	NT_022300:33	NM_032788	1395	ZnF-C2H2	zinc finger protein 514
ZNF519	NT_010859:601	NM_145287	1396	ZnF-C2H2	zinc finger protein 519
ZNF528	NT_011109:1343	NM_032423	1397	ZnF-C2H2	zinc finger protein 528
ZNF6		NM_021998	1398	ZnF-C2H2	zinc finger protein 6 (CMPX1)
ZNF7		NM_003416	1399	ZnF-C2H2	zinc finger protein 7 (KOX 4, clone HF.16)
ZNF71	NT_011104:94	NM_021216	1400	ZnF-C2H2	zinc finger protein 71 (Cos26)
ZNF73		NM_012480	1401	ZnF-C2H2	zinc finger protein 73 (Cos12)
ZNF74		NM_003426	1402	ZnF-C2H2	zinc finger protein 74 (Cos52)
ZNF75	NT_011786:383	NM_007131	1403	ZnF-C2H2	zinc finger protein 75 (D8C6)
ZNF75A		NM_153028	1404	ZnF-C2H2	zinc finger protein 75a

Gene Abbrev	ScriptSureID	mRNA ID	SEQ ID NO:	Class	Description
ZNF76		NM_003427	1405	ZnF-C2H2	zinc finger protein 76 (expressed in testis)
ZNF77	NT_011255:4	NM_021217	1406	ZnF-C2H2	zinc finger protein 77 (pT1)
ZNF79		NM_007135	1407	ZnF-C2H2	zinc finger protein 79 (pT7)
ZNF8		M29581	1408	ZnF-C2H2	zinc-finger protein 8 (clone HF.18)
ZNF80		NM_007136	1409	ZnF-C2H2	zinc finger protein 80 (pT17)
ZNF81		X68011	1410	ZnF-C2H2	zinc finger protein 81 (HFZ20)
ZNF83		NM_018300	1411	ZnF-C2H2	zinc finger protein 83 (HPF1)
ZNF84		NM_003428	1412	ZnF-C2H2	zinc finger protein 84 (HPF2)
ZNF85		NM_003429	1413	ZnF-C2H2	zinc finger protein 85 (HPF4, HTF1)
ZNF9		NM_003418	1414	ZnF-Other	zinc finger protein 9 (a cellular retroviral nucleic acid binding protein)
ZNF90		M61870	1415	ZnF-C2H2	zinc finger protein 90 (HTF9)
ZNF91		NM_003430	1416	ZnF-C2H2	zinc finger protein 91 (HPF7, HTF10)
ZNF92		M61872	1417	ZnF-C2H2	zinc finger protein 92 (HTF12)
ZNF93		M61873	1418	ZnF-C2H2	zinc finger protein 93 (HTF34)
ZNF-kaiso		NM_006777	1419	ZnF-BTB/POZ	Kaiso
ZNFN1A1		NM_006060	1420	ZnF-C2H2	zinc finger protein, subfamily 1A, 1 (Ikaros)
ZNFN1A2		NM_016260	1421	ZnF-C2H2	zinc finger protein, subfamily 1A, 2 (Helios)
ZNFN1A3		NM_012481	1422	ZnF-C2H2	zinc finger protein, subfamily 1A, 3 (Aiolos)
ZNFN1A4	NT_009458:35	NM_022465	1423	ZnF-MYND	zinc finger protein, subfamily 1A, 4 (Eos)
ZNF-U69274		NM_014415	1424	ZnF-BTB/POZ	zinc finger protein
ZNRF1	NT_035368:168	NM_032268	1425	ZnF-Other	zinc and ring finger protein 1
ZXDA		L14787	1426	ZnF-C2H2	zinc finger, X-linked, duplicated A
ZXDB		L14788	1427	ZnF-C2H2	zinc finger, X-linked, duplicated B
ZYX	NT_007914:428	NM_003461	1428	Co-activator	zyxin

[00451] SOX2 (SEQ ID NO: 1501; NM_003106)

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1621 gagagagatc ctggacttct ttttgggggga ctatttttgt acagagaaaa cctggggagg
1681 gtggggaggg cgggggaatg gaccttgtat agatctggag gaaagaaagc tacgaaaaac
1741 tttttaaaag ttctagtggt acggtaggag ctttgcagga agtttgcaaa agtctttacc
1801 aataatattt agagctagtc tccaagcgac gaaaaaaatg ttttaatatt tgcaagcaac
1861 ttttgtacag tatttatcga gataaacatg gcaatcaaaa tgtccattgt ttataagctg
1921 agaatttgcc aatatttttc aaggagaggc ttcttgctga attttgattc tgcagctgaa
1981 atttaggaca gttgcaaacg tgaaaagaag aaaattattc aaatttggac attttaattg
2041 tttaaaaatt gtacaaaagg aaaaaattag aataagtact ggcgaaccat ctctgtggtc
2101 ttgtttaaaa agggcaaaag ttttagactg tactaaattt tataacttac tgttaaaagc
2161 aaaaatggcc atgcaggttg acaccgttgg taatttataa tagcttttgt tcgatcccaa
2221 ctttccattt tgttcagata aaaaaaacca tgaaattact gtgtttgaaa tattttctta
2281 tggtttgtaa tatttctgta aatttattgt gatattttaa ggttttcccc cctttatttt
2341 ccgtagttgt attttaaaag attcggctct gtattatttg aatcagtctg ccgagaatcc
2401 atgtatatat ttgaactaat atcatcctta taacaggtac attttcaact taagttttta
2461 ctccattatg cacagtttga gataaataaa tttttgaaat atggacactg aaaaaaaaaa
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FoxP3

[00452] The FOXP3 (forkhead box P3) gene encodes for a protein involved in immune system responses. A member of the FOX protein family, FOXP3 is a transcription factor that plays a role in the development and function of regulatory T cells. The induction or administration of Foxp3 positive T cells in animal studies indicate marked reductions in (autoimmune) disease severity in models of diabetes, multiple sclerosis, asthma, inflammatory bowel disease, thyroiditis and renal disease.

[00453] The FoxP3 protein can be expressed in a cell using the synthetic, modified RNAs described herein.

Targeting moiety

[00454] As used herein, the term "targeting moiety" refers to an agent that directs a composition to a particular tissue, cell type, receptor, or other area of interest. As per this definition, a targeting moiety can be attached directly to a synthetic, modified RNA or indirectly to a composition used for delivering a synthetic, modified RNA (*e.g.*, a liposome, polymer etc) to direct expression in a

particular cell etc. A targeting moiety can also be encoded or expressed by a synthetic, modified-NA as described herein, such that a cell expresses a targeting moiety on it surface, permitting a cell to be targeted to a desired tissue, organ etc. For the avoidance of confusion, targeting moieties expressed on a cell surface are referred to herein as "homing moieties."

[00455] Non-limiting examples of a targeting moiety or homing moiety include, but are not limited to, an oligonucleotide, an antigen, an antibody or functional fragment thereof, a ligand, a cell-surface receptor, a membrane-bound molecule, one member of a specific binding pair, a polyamide including a peptide having affinity for a biological receptor, an oligosaccharide, a polysaccharide, a steroid or steroid derivative, a hormone, *e.g.*, estradiol or histamine, a hormone-mimic, *e.g.*, morphine, or hormone-receptor, or other compound having binding specificity for a target. In the methods of the present invention, a targeting moiety promotes transport or preferential localization of a synthetic, modified RNA to a target cell, while a homing moiety permits the targeting of a cell modified using the synthetic, modified RNAs described herein to a particular tissue *in vivo*. It is contemplated herein that the homing moiety can be also encoded in a cell by a synthetic, modified RNA as described herein.

[00456] A synthetic, modified RNA or composition thereof can be targeted by means of a targeting moiety, including, *e.g.*, an antibody or targeted liposome technology. In some embodiments, a synthetic, modified RNA or composition thereof is targeted to a specific tissue by using bispecific antibodies, for example produced by chemical linkage of an anti-ligand antibody (Ab) and an Ab directed toward a specific target. To avoid the limitations of chemical conjugates, molecular conjugates of antibodies can be used for production of recombinant, bispecific single-chain Abs directing ligands and/or chimeric inhibitors at cell surface molecules. The addition of an antibody to a synthetic, modified RNA composition permits the agent attached to accumulate additively at the desired target site. Antibody-based or non- antibody-based targeting moieties can be employed to deliver a ligand or the inhibitor to a target site. Preferably, a natural binding agent for an unregulated or disease associated antigen is used for this purpose.

[00457] **Table 2** and **Table 3** provide non-limiting examples of CD ("cluster of differentiation") molecules and other cell-surface/membrane bound molecules and receptors, such as transmembrane tyrosine kinase receptors, ABC transporters, and integrins, that can be expressed using the synthetic, modified RNA compositions and methods described herein for targeting and homing to cells of interest, or for changing the phenotype of a cell.

Table 2: List of CD Molecules

Molecule (CD Number)	NCBI Name	NCBI Other Names
CD10	MME	CALLA; CD10; NEP
CD100	SEMA4D	CD100; M-sema G; M-sema-G; SEMAJ; coll-4
CD101	IGSF2	CD101; V7
CD102	ICAM2	CD102

Molecule (CD Number)	NCBI Name	NCBI Other Names
CD103	ITGAE	CD103; HUMINAE
CD104	ITGB4	
CD105	ENG	CD105; END; HHT1; ORW; ORW1
CD106	VCAM1	INCAM-100
CD107a	LAMP1	CD107a; LAMPA; LGP120
CD107b	LAMP2	CD107b; LAMPB
CD107b	LAMP2	CD107b; LAMPB
CD108	SEMA7A	CD108; CDw108; H-SEMA-K1; H-Sema K1; H-Sema-L; SEMAK1; SEMAL
CD109	CD109	DKFZp762L1111; FLJ38569
CD110	MPL	C-MPL; CD110; MPLV; TPOR
CD111	PVRL1	CD111; CLPED1; ED4; HIgR; HVEC; PRR; PRR1; PVRR; PVRR1; SK-12
CD112	PVRL2	CD112; HVEB; PRR2; PVRR2
CD113	PVRL3	PVTL3; PPR3; PRR3; PVRR3; nectin-3; DKFZP566B0846
CD114	CSF3R	CD114; GCSFR
CD115	CSF1R	C-FMS; CD115; CSFR; FIM2; FMS
CD116	CSF2RA	CD116; CDw116; CSF2R; CSF2RAX; CSF2RAY; CSF2RX; CSF2RY; GM-CSF-R-alpha; GMCSFR; GMR; MGC3848; MGC4838
CD117	KIT	CD117; PBT; SCFR
CD118	LIFR	LIFR; SWS; SJS2; STWS
CD119	IFNGR1	CD119; IFNGR
CD11a	ITGAL	CD11A; LFA-1; LFA1A
CD11a	ITGAL	CD11A; LFA-1; LFA1A
CD11a	ITGAL	CD11A; LFA-1; LFA1A
CD11b	ITGAM	CD11B; CR3A; MAC-1; MAC1A; MO1A
CD11c	ITGAX	CD11C
CD11d	ITGAD	ADB2; CD11D
CD120a	TNFRSF1A	CD120a; FPF; MGC19588; TBP1; TNF-R; TNF-R-I; TNF-R55; TNFAR; TNFR1; TNFR55; TNFR60; p55; p55-R; p60
CD120b	TNFRSF1B	CD120b; TBPII; TNF-R-II; TNF-R75; TNFBR; TNFR2; TNFR80; p75; p75TNFR
CD121a	IL1R1	CD121A; D2S1473; IL-1R-alpha; IL1R; IL1RA; P80
CD121b	IL1R2	IL1RB; MGC47725
CD122	IL2RB	P70-75
CD123	IL3RA	CD123; IL3R; IL3RAY; IL3RX; IL3RY; MGC34174; hIL-3Ra
CD124	IL4R	CD124; IL4RA
CD125	IL5RA	CDw125; HSIL5R3; IL5R; MGC26560
CD126	IL6R	CD126; IL-6R-1; IL-6R-alpha; IL6RA
CD127	IL7R	CD127; CDW127; IL-7R-alpha
CD128a	see CD181	see CD181
CD128b	see CD182	see CD182
CD129	IL9R	
CD13	ANPEP	CD13; LAP1; PEPN; gp150
CD130	IL6ST	CD130; CDw130; GP130; GP130-RAPS; IL6R-beta
CD131	CSF2RB	CD131; CDw131; IL3RB; IL5RB
CD132	IL2RG	CD132; IMD4; SCIDX; SCIDX1
CD133	PROM1	AC133; CD133; PROML1
CD134	TNFRSF4	ACT35; CD134; OX40; TXGP1L
CD135	FLT3	CD135; FLK2; STK1
CD136	MST1R	CDw136; RON
CD137	TNFRSF9	4-1BB; CD137; CDw137; ILA; MGC2172
CD138	SDC1	CD138; SDC; SYND1
CD139	CD139	
CD14	CD14	
CD14	CD14	
CD140a	PDGFRA	CD140A; PDGFR2
CD140b	PDGFRB	CD140B; JTK12; PDGF-R-beta; PDGFR; PDGFR1

Molecule (CD Number)	NCBI Name	NCBI Other Names
CD141	THBD	CD141; THRM; TM
CD142	F3	CD142; TF; TFA
CD143	ACE	ACE1; CD143; DCP; DCP1; MGC26566
CD144	CDH5	7B4
CD146	MCAM	CD146; MUC18
CD147	BSG	5F7; CD147; EMMPRIN; M6; OK; TCSF
CD148	PTPRJ	CD148; DEP1; HPTPeta; R-PTP-ETA; SCC1
CD149	see CD47R	see CD47R
CD15	FUT4	CD15; ELFT; FCT3A; FUC-TIV
CD15	FUT4	CD15; ELFT; FCT3A; FUC-TIV
CD15	FUT4	CD15; ELFT; FCT3A; FUC-TIV
CD150	SLAMF1	CD150; CDw150; SLAM
CD151	CD151	GP27; PETA-3; SFA1
CD152	CTLA4	CD152
CD153	TNFSF8	CD153; CD30L; CD30LG
CD154	CD40LG	CD154; CD40L; CD40LG; HIGM1; IGM; IMD3; T-BAM; TRAP; gp39; hCD40L
CD155	PVR	CD155; HVED; NECL5; PVS; TAGE4
CD156a	ADAM8	CD156; MS2
CD156b	ADAM17	CD156b; TACE; cSVP
CD156C	ADAM10	kuz; MADM; CD156c; HsT18717
CD157	BST1	CD157
CD158A	KIR2DL1	47.11; CD158A; CL-42; NKAT1; p58.1
CD158B1	KIR2DL2	CD158B1; CL-43; NKAT6; p58.2
CD158B2	KIR2DL3	CD158B2; CD158b; CL-6; KIR-023GB; NKAT2; NKAT2A; NKAT2B; p58
CD158C	KIR3DP1; KIR2DS6; KIRX	LOC392419
CD158D	KIR2DL4	103AS; 15.212; CD158D; KIR103; KIR103AS
CD158E1	KIR3DL1	AMB11; CD158E1; CD158E1/2; CD158E2; CL-11; CL-2; KIR; KIR3DS1; NKAT10; NKAT3; NKB1; NKB1B
CD158E2	KIR3DS1	AMB11; CD158E1; CD158E1/2; CD158E2; CL-11; CL-2; KIR; KIR3DS1; NKAT10; NKAT3; NKB1; NKB1B
CD158F	KIR2DL5	CD158F; KIR2DL5; KIR2DL5.1; KIR2DL5.3
CD158G	KIR2DS5	CD158G; NKAT9
CD158H	KIR2DS1	CD158H; EB6ActI; EB6ActII; p50.1
CD158I	KIR2DS4	CD158I; KIR1D; KKA3; NKAT8; PAX; cl-39
CD158J	KIR2DS2	183ACTI; CD158J; CL-49; NKAT5; p50.2
CD158K	KIR3DL2	CD158K; CL-5; NKAT4; NKAT4A; NKAT4B
CD159a	KLRC1	CD159A; MGC13374; MGC59791; NKG2; NKG2A
CD159c	KLRC2	
CD160	CD160	BY55; NK1; NK28
CD161	KLRB1	CD161; NKR; NKR-P1; NKR-P1A; NKRP1A; hNKR-P1A
CD162	SELPLG	CD162; PSGL-1; PSGL1
CD163	CD163	M130; MM130
CD164	CD164	MGC-24; MUC-24; endolyn
CD165	CD165	
CD166	ALCAM	CD166; MEMD
CD167a	DDR1	CAK; CD167; DDR; EDDR1; MCK10; NEP; NTRK4; PTK3; PTK3A; RTK6; TRKE
CD167b	DDR2	TKT; MIG20a; NTRKR3; TYRO10
CD168	HMMR	RHAMM
CD169	SN	CD169; FLJ00051; FLJ00055; FLJ00073; FLJ32150; SIGLEC-1; dJ1009E24.1
CD16a	FCGR3A	CD16; FCG3; FCGR3; IGFR3
CD16b	FCGR3B	CD16; FCG3; FCGR3
CD17	carbohydrate	carbohydrate

Molecule (CD Number)	NCBI Name	NCBI Other Names
CD170	SIGLEC5	CD33L2; OB-BP2; OBBP2; SIGLEC-5
CD171	L1CAM	CAML1; CD171; IISAS; IISAS1; MASA; MIC5; N-CAML1; S10; SPG1
CD172a	PTPNS1	BIT; MFR; MYD-1; P84; SHPS-1; SHPS1; SIRP; SIRP-ALPHA-1; SIRPalpha; SIRPalpha2
CD172b	SIRPB1	SIRP-BETA-1
CD172g	SIRPB2	SIRP-B2; bA77C3.1
CD173	carbohydrate	carbohydrate
CD174	FUT3	LE; Les
CD175	carbohydrate	carbohydrate
CD175s	carbohydrate	carbohydrate
CD176	carbohydrate	carbohydrate
CD177	CD177	CD177; HNA2A; NB1
CD178	FASLG	FASL; CD178; CD95L; TNFSF6; APT1LG1
CD179a	VPREB1	IGI; IGVPB; VPREB
CD179b	IGLL1	14.1; CD179b; IGL1; IGL5; IGLL; IGO; IGVPB; VPREB2
CD18	ITGB2	CD18; LAD; LCAMB; LFA-1; MF17; MFI7
CD180	CD180	LY64; Ly78; RP105; MGC126233; MGC126234
CD181	IL8RA	C-C CKR-1; C-C-CKR-1; CD128; CDw128a; CMKAR1; CXCR1; IL8R1; IL8RBA
CD182	IL8RB	CDw128b; CMKAR2; CXCR2; IL8R2; IL8RA
CD183	CXCR3	CD183; CKR-L2; CMKAR3; GPR9; IP10; IP10-R; Mig-R; MigR
CD184	CXCR4	D2S201E; HM89; HSY3RR; LAP3; LESTR; NPY3R; NPYR; NPYY3R; WHIM
CD185	BLR1	BLR1; CXCR5; MDR15
CD186	CXCR6	CXCR6; BONZO; STRL33; TYMSTR
CD187		
CD188		
CD189		
CD19	CD19	B4; MGC12802
CD190		
CD191	CCR1	CKR-1; CMKBR1; HM145; MIP1aR; SCYAR1
CD192	CCR2	CC-CKR-2; CCR2A; CCR2B; CKR2; CKR2A; CKR2B; CMKBR2; MCP-1-R
CD193	CCR3	CC-CKR-3; CKR3; CMKBR3
CD194	CCR4	CC-CKR-4; CKR4; CMKBR4; ChemR13; HGCN
CD195	CCR5	CC-CKR-5; CCCKR5; CD195; CKR-5; CKR5; CMKBR5
CD196	CCR6	CCR6; BN-1; CKR6; DCR2; CKRL3; DRY-6; GPR29; CKR-L3; CMKBR6; GPRCY4; STRL22; GPR-CY4
CD197	CCR7	BLR2; CDw197; CMKBR7; EBI1
CD1a	CD1A	CD1
CD1b	CD1B	CD1
CD1c	CD1C	CD1
CD1d	CD1D	
CD1d	CD1D	
CD1e	CD1E	HSCDIEL
CD2	CD2	SRBC; T11
CD2	CD2	SRBC; T11
CD20	MS4A1	B1; Bp35; CD20; LEU-16; MGC3969; MS4A2; S7
CD200	CD200	MOX1; MOX2; MRC; OX-2
CD201	PROCR	CCCA; CCD41; EPCR; MGC23024; bA42O4.2
CD202b	TEK	CD202B; TIE-2; TIE2; VMCM; VMCM1
CD203c	ENPP3	B10; CD203c; NPP3; PD-IBETA; PDNP3
CD204	MSR1	SCARA1; SR-A; phSR1; phSR2
CD205	LY75	CLEC13B; DEC-205; GP200-MR6
CD206	MRC1	CLEC13D
CD207	CD207	LANGERIN
CD208	LAMP3	DC-LAMP; DCLAMP; LAMP; TSC403

Molecule (CD Number)	NCBI Name	NCBI Other Names
CD209	CD209	CDSIGN; DC-SIGN; DC-SIGN1
CD21	CR2	C3DR; CD21
CD211		
CD212	IL12RB1	IL-12R-BETA1; IL12RB; MGC34454
CD213a1	IL13RA1	IL-13Ra; NR4
CD213a2	IL13RA2	IL-13R; IL13BP
CD214		
CD215		
CD216		
CD217	IL17R	IL-17RA; IL17RA; MGC10262; hIL-17R
CD218a	IL18R1	IL18R1; IL1RRP; IL-1Rrp
CD218b	IL18RAP	IL18RAP; ACPL
CD219		
CD22	CD22	SIGLEC-2
CD220	INSR	
CD221	IGF1R	JTK13
CD222	IGF2R	CD222; CIMPR; M6P-R; MPRI
CD223	LAG3	CD223
CD224	GGT1	CD224; D22S672; D22S732; GGT; GTG
CD225	IFITM1	Sep-27; CD225; IFI17; LEU13
CD226	CD226	DNAM-1; DNAM1; PTA1; TLiSA1
CD227	MUC1	CD227; EMA; PEM; PUM
CD228	MFI2	MAP97; MGC4856; MTF1
CD229	LY9	CD229; SLAMF3; hly9; mLY9
CD23	FCER2	CD23; CD23A; FCE2; IGEBF
CD230	PRNP	ASCR; CJD; GSS; MGC26679; PRIP; PrP; PrP27-30; PrP33-35C; PrPc
CD231	TSPAN7	A15; CCG-B7; CD231; DXS1692E; MXS1; TALLA-1; TM4SF2b
CD232	PLXNC1	PLXN-C1; VESPR
CD233	SLC4A1	AE1; BND3; CD233; DI; EMPB3; EPB3; RTA1A; WD; WD1
CD234	DARC	CCBP1; DARC; GPD
CD235a	GYPA	GPA; MN; MNS
CD235b	GYPB	GPB; MNS; SS
CD236	GYPC	GE; GPC
CD237		
CD238	KEL	
CD239	LU	AU; BCAM; MSK19
CD24	CD24	CD24A
CD240CE	RHCE	RH; RH30A; RHC; RHE; RHIXB; RHPI; Rh4; RhVI; RhVIII
CD240D	RHD	CD240D; DIIIc; RH; RH30; RHCED; RHDVA(TT); RHPII; RHXIII; Rh30a; Rh4; RhII; RhK562-II; RhPI
CD241	RHAG	RH2; RH50A
CD242	ICAM4	LW
CD243	ABCB1	ABC20; CD243; CLCS; GP170; MDR1; P-gp; PGY1
CD244	CD244	2B4; NAIL; NKR2B4; Nmrk; SLAMF4
CD245	CD245	
CD246	ALK	
CD247	CD247	CD3-ZETA; CD3H; CD3Q; TCRZ
CD248	CD248	CD164L1
CD249	ENPEP	APA; gp160; EAP
CD25	IL2RA	CD25; IL2R; TCGFR
CD25	IL2RA	CD25; IL2R; TCGFR
CD25	IL2RA	CD25; IL2R; TCGFR
CD25	IL2RA	CD25; IL2R; TCGFR
CD25	IL2RA	CD25; IL2R; TCGFR
CD250		

Molecule (CD Number)	NCBI Name	NCBI Other Names
CD251		
CD252	TNFSF4	TNFSF4; GP34; OX4OL; TXGP1; CD134L; OX-40L; OX40L
CD253	TNFSF10	TNFSF10; TL2; APO2L; TRAIL; Apo-2L
CD254	TNFSF11	ODF; OPGL; sOdf; CD254; OPTB2; RANKL; TRANCE; hRANKL2
CD255		
CD256	TNFSF13	APRIL; TALL2; TRDL-1; UNQ383/PRO715
CD257	TNFSF13B	BAFF; BLYS; TALL-1; TALL1; THANK; TNFSF20; ZTNF4; delta BAFF
CD258	TNFSF14	TNFSF14; LTg; TR2; HVEML; LIGHT
CD259		
CD26	DPP4	ADABP; ADCP2; CD26; DPPIV; TP103
CD260		
CD261	TNFRSF10A	APO2; DR4; MGC9365; TRAILR-1; TRAILR1
CD262	TNFRSF10B	DR5; KILLER; KILLER/DR5; TRAIL-R2; TRAILR2; TRICK2; TRICK2A; TRICK2B; TRICKB; ZTNFR9
CD263	TNFRSF10C	DCR1; LIT; TRAILR3; TRID
CD264	TNFRSF10D	DCR2; TRAILR4; TRUNDD
CD265	TNFRSF11A	EOF; FEO; ODFR; OFE; PDB2; RANK; TRANCER
CD266	TNFRSF12A	TNFRSF12A; FN14; TWEAKR
CD267	TNFRSF13B	CVID; TACI; CD267; FLJ39942; MGC39952; MGC133214; TNFRSF14B
CD268	TNFRSF13C	BAFFR; CD268; BAFF-R; MGC138235
CD269	TNFRSF17	BCM; BCMA
CD27	TNFRSF7	CD27; MGC20393; S152; T14; Tp55
CD270		
CD271	NGFR	NGFR; TNFRSF16; p75(NTR)
CD272	BTLA	BTLA1; FLJ16065
CD273	PDCD1LG2	PDCD1LG2; B7DC; Btdc; PDL2; PD-L2; PDCD1L2; bA574F11.2
CD274	CD274	B7-H; B7H1; PD-L1; PDCD1L1; PDL1
CD275	ICOSLG	B7-H2; B7H2; B7RP-1; B7RP1; GL50; ICOS-L; ICOSLG; KIAA0653; LICOS
CD276	CD276	B7H3
CD277	BTN3A1	BTF5; BT3.1
CD278	ICOS	AILIM; MGC39850
CD279	PDCD1	PD1; SLEB2; hPD-1
CD28	CD28	Tp44
CD28	CD28	Tp44
CD28	CD28	Tp44
CD28	CD28	Tp44
CD28	CD28	Tp44
CD28	CD28	Tp44
CD280	MRC2	MRC2; UPARAP; ENDO180; KIAA0709
CD281	TLR1	TLR1; TIL; rsc786; KIAA0012; DKFZp547I0610; DKFZp564I0682
CD282	TLR2	TIL4
CD283	TLR3	TLR3
CD284	TLR4	TOLL; hToll
CD285		
CD286	TLR6	CD286
CD287		
CD288	TLR8	TLR8
CD289	TLR9	none
CD29	ITGB1	CD29; FNRB; GPIIA; MDF2; MSK12; VLAB
CD290	TLR10	TLR10
CD291		
CD292	BMPR1A	BMPR1A; ALK3; ACVRLK3
CD294	GPR44	CRTH2
CD295	LEPR	LEPR; OBR
CD296	ART1	ART2; RT6

Molecule (CD Number)	NCBI Name	NCBI Other Names
CD297	ART4	DO; DOK1; CD297; ART4
CD298	ATP1B3	ATP1B3; ATPB-3; FLJ29027
CD299	CLEC4M	DC-SIGN2; DC-SIGNR; DCSIGNR; HP10347; LSIGN; MGC47866
CD3	see CD3D, CD3E, CD3G	see CD3D, CD3E, CD3G
CD3	see CD3D, CD3E, CD3G	see CD3D, CD3E, CD3G
CD30	TNFRSF8	CD30; D1S166E; KI-1
CD300a	CD300A	CMRF-35-H9; CMRF35H; CMRF35H9; IRC1; IRC2; IRp60
CD300C	CD300C	CMRF-35A; CMRF35A; CMRF35A1; LIR
CD301	CLEC10A	HML; HML2; CLECSF13; CLECSF14
CD302	CD302	DCL-1; BIMLEC; KIAA0022
CD303	CLEC4C	BDCA2; CLECSF11; DLEC; HECL; PRO34150; CLECSF7
CD304	NRP1	NRP; VEGF165R
CD305	LAIR1	LAIR-1
CD306	LAIR2	LAIR2
CD307	FCRL5	BXMAS1
CD308		
CD309	KDR	KDR; FLK1; VEGFR; VEGFR2
CD31	PECAM1	CD31
CD31	PECAM1	CD31
CD31	PECAM1	CD31
CD310		
CD311		
CD312	EMR2	
CD313		
CD314	KLRK1	KLRK1; KLR; NKG2D; NKG2-D; D12S2489E
CD315	PTGFRN	PTGFRN; FPRP; EWI-F; CD9P-1; SMAP-6; FLJ11001; KIAA1436
CD316	IGSF8	IGSF8; EWI2; PGRL; CD81P3
CD317	BST2	none
CD318	CDCP1	CDCP1; FLJ22969; MGC31813
CD319	SLAMF7	19A; CRACC; CS1
CD320	CD320	8D6A; 8D6
CD321	F11R	JAM; KAT; JAM1; JCAM; JAM-1; PAM-1
CD322	JAM2	C21orf43; VE-JAM; VEJAM
CD323		
CD324	CDH1	Arc-1; CDHE; ECAD; LCAM; UVO
CD325	CDH2	CDHN; NCAD
CD326	TACSTD1	CO17-1A; EGP; EGP40; Ep-CAM; GA733-2; KSA; M4S1; MIC18; MK-1; TROP1; hEGP-2
CD327	SIGLEC6	CD33L; CD33L1; OBBP1; SIGLEC-6
CD328	SIGLEC7	p75; QA79; AIRM1; CDw328; SIGLEC-7; p75/AIRM1
CD329	SIGLEC9	CDw329; OBBP-LIKE
CD32a	FCGR2A	CD32; CDw32; FCG2; FCGR2; FCGR2A1; FcGR; IGFR2; MGC23887; MGC30032
CD32b	FCGR2B	CD32; FCG2; FCGR2; IGFR2
CD32c	FCGR2C	CD32; FcgammaRIIC
CD33	CD33	SIGLEC-3; p67
CD33	CD33	SIGLEC-3; p67
CD330		
CD331	FGFR1	FGFR1; H2; H3; H4; H5; CEK; FLG; FLT2; KAL2; BFGFR; C-FGR; N-SAM
CD332	FGFR2	FGFR2; BEK; JWS; CEK3; CFD1; ECT1; KGFR; TK14; TK25; BFR-1; K-SAM
CD333	FGFR3	FGFR3; ACH; CEK2; JTK4; HSFGFR3EX
CD334	FGFR4	FGFR4; TKF; JTK2; MGC20292
CD335	NCR1	LY94; NK-p46; NKP46

Molecule (CD Number)	NCBI Name	NCBI Other Names
CD336	NCR2	LY95; NK-p44; NKP44
CD337	NCR3	1C7; LY117; NKp30
CD338	ABCG2	MRX; MXR; ABCP; BCRP; BMDP; MXR1; ABC15; BCRP1; CDw338; EST157481; MGC102821
CD339	JAG1	JAG1; AGS; AHD; AWS; HJ1; JAGL1
CD34	CD34	
CD34	CD34	
CD340	ERBB2	NEU; NGL; HER2; TKR1; HER-2; c-erb B2; HER-2/neu
CD344	FZD4	EVR1; FEVR; Fz-4; FzE4; GPCR; FZD4S; MGC34390
CD349	FZD9	FZD3
CD35	CR1	C3BR; CD35
CD350	FZD10	FzE7; FZ-10; hFz10
CD36	CD36	FAT; GP3B; GP4; GPIV; PASIV; SCARB3
CD37	CD37	GP52-40
CD38	CD38	T10
CD39	ENTPD1	ATPDase; CD39; NTPDase-1
CD3d	CD3D	CD3-DELTA; T3D
CD3e	CD3E	CD3-EPSILON; T3E; TCRE
CD3g	CD3G	CD3-GAMMA; T3G
CD4	CD4	
CD4	CD4	
CD40	CD40	p50; Bp50; CDW40; MGC9013; TNFRSF5
CD41	ITGA2B	CD41; CD41B; GP2B; GPIIb; GTA
CD42a	GP9	CD42a
CD42b	GP1BA	BSS; CD42B; CD42b-alpha; GP1B; MGC34595
CD42c	GP1BB	CD42c
CD42d	GP5	CD42d
CD43	SPN	CD43; GPL115; LSN
CD43	SPN	CD43; GPL115; LSN
CD43	SPN	CD43; GPL115; LSN
CD43	SPN	CD43; GPL115; LSN
CD44	CD44	CDW44; ECMR-III; IN; INLU; LHR; MC56; MDU2; MDU3; MGC10468; MIC4; MUTCH-I; Pgp1
CD44	CD44	CDW44; ECMR-III; IN; INLU; LHR; MC56; MDU2; MDU3; MGC10468; MIC4; MUTCH-I; Pgp1
CD44	CD44	CDW44; ECMR-III; IN; INLU; LHR; MC56; MDU2; MDU3; MGC10468; MIC4; MUTCH-I; Pgp1
CD45	PTPRC	B220; CD45; GP180; LCA; LY5; T200
CD45RA	PTPRC	
CD45RB	PTPRC	
CD45RC	PTPRC	
CD45RO	PTPRC	
CD46	MCP	CD46; MGC26544; MIC10; TLX; TRA2.10
CD47	CD47	IAP; MER6; OA3
CD48	CD48	BCM1; BLAST; BLAST1; MEM-102; SLAMF2; hCD48; mCD48
CD49a	ITGA1	CD49a; VLA1
CD49b	ITGA2	BR; CD49B; VLAA2
CD49c	ITGA3	CD49C; GAP-B3; GAPB3; MSK18; VCA-2; VL3A; VLA3a
CD49d	ITGA4	CD49D
CD49e	ITGA5	CD49e; FNRA; VLA5A
CD49f	ITGA6	CD49f
CD5	CD5	LEU1; T1
CD5	CD5	LEU1; T1
CD50	ICAM3	CD50; CDW50; ICAM-R
CD51	ITGAV	CD51; MSK8; VNRA

Molecule (CD Number)	NCBI Name	NCBI Other Names
CD52	CD52	CD52
CD53	CD53	MOX44
CD54	ICAM1	BB2; CD54
CD55	DAF	CD55; CR; TC
CD56	NCAM1	CD56; MSK39; NCAM
CD57	CD57	HNK-1; LEU7; NK-1
CD58	CD58	LFA3
CD59	CD59	MGC2354; MIC11; MIN1; MIN2; MIN3; MSK21; PROTECTIN
CD6	CD6	TP120
CD6	CD6	TP120
CD60a	carbohydrate	carbohydrate
CD60b	carbohydrate	carbohydrate
CD60b	carbohydrate	carbohydrate
CD60c	carbohydrate	carbohydrate
CD61	ITGB3	CD61; GP3A; GPIIIa
CD62E	SELE	CD62E; ELAM; ELAM1; ESEL; LECAM2
CD62L	SELL	CD62L; LAM-1; LAM1; LECAM1; LNHR; LSEL; LYAM1; Leu-8; Lyam-1; PLNHR; TQ1; hLHRc
CD62P	SELP	CD62; CD62P; GMP140; GRMP; PADGEM; PSEL
CD63	CD63	LAMP-3; ME491; MLA1; OMA81H
CD64a	FCGR1A	CD64; FCRI; IGFR1
CD65	carbohydrate	carbohydrate
CD65s	carbohydrate	carbohydrate
CD66a	CEACAM1	BGP; BGP1; BGPI; CD66; CD66A
CD66b	CEACAM8	CD66b; CD67; CGM6; NCA-95
CD66c	CEACAM6	CD66c; CEAL; NCA
CD66d	CEACAM3	CD66D; CGM1
CD66e	CEACAM5	CD66e; CEA
CD66f	PSG1	B1G1; CD66f; PBG1; PSBG1; PSGGA; SP1
CD67	see CD66f	see CD66f
CD68	CD68	SCARD1
CD69	CD69	none
CD7	CD7	GP40; LEU-9; TP41; Tp40
CD7	CD7	GP40; LEU-9; TP41; Tp40
CD70	TNFSF7	CD27L; CD27LG; CD70
CD71	TFRC	CD71; TFR; TRFR
CD72	CD72	LYB2
CD73	NT5E	CD73; E5NT; NT5; NTE; eN; eNT
CD74	CD74	DHLAG; HLADG; Ia-GAMMA
CD75	carbohydrate	carbohydrate
CD75s	carbohydrate	carbohydrate
CD76	see CD75 and CD75s	see CD75 and CD75s
CD77	carbohydrate	carbohydrate
CD78	deleted	deleted
CD79a	CD79A	IGA; MB-1
CD79b	CD79B	B29; IGB
CD80	CD80	CD28LG; CD28LG1; LAB7
CD81	CD81	S5.7; TAPA1
CD82	CD82	4F9; C33; CD82; GR15; IA4; R2; SAR2; ST6
CD83	CD83	BL11; HB15
CD84	CD84	LY9B; SLAMF5; hCD84; mCD84
CD85A	LILRB3	CD85A; HL9; ILT5; LIR-3; LIR3
CD85B	LILRB6	LILRB6
CD85C	LILRB5	CD85C; LIR-8; LIR8

Molecule (CD Number)	NCBI Name	NCBI Other Names
CD85D	LILRB2	CD85D; ILT4; LIR-2; LIR2; MIR-10; MIR10
CD85E	LILRA3	CD85E; HM31; HM43; ILT6; LIR-4; LIR4
CD85F	LILRB7	CD85F; ILT11; LILRB7
CD85G	LILRA4	ILT7; CD85g; MGC129597
CD85H	LILRA2	CD85H; ILT1; LIR-7; LIR7
CD85I	LILRA1	CD85I; LIR-6; LIR6
CD85J	LILRB1	CD85; CD85J; ILT2; LIR-1; LIR1; MIR-7; MIR7
CD85K	LILRB4	CD85K; HM18; ILT3; LIR-5; LIR5
CD85L	LILRP1	ILT9; CD85l; LILRA6P
CD85M	LILRP2	CD85m; ILT10; LILRA5
CD86	CD86	B7-2; B70; CD28LG2; LAB72; MGC34413
CD87	PLAUR	CD87; UPAR; URKR
CD88	C5R1	C5A; C5AR; CD88
CD89	FCAR	CD89
CD8a	CD8A	CD8; Leu2; MAL; p32
CD8a	CD8A	CD8; Leu2; MAL; p32
CD8b	CD8B1	CD8B; LYT3; Leu2; Ly3
CD9	CD9	BA2; DRAP-27; MIC3; MRP-1; P24
CD90	THY1	CD90
CD91	LRP1	A2MR; APOER; APR; CD91; LRP
CD92	SLC44A1	CTL1; CDW92; CHTL1; RP11-287A8.1
CD93	CD93	C1QR1; C1qRP; CDw93; MXRA4; C1qR(P); dJ737E23.1
CD94	KLRD1	CD94
CD95	FAS	APT1; CD95; FAS1; APO-1; FASTM; ALPS1A; TNFRSF6
CD96	CD96	MGC22596; TACTILE
CD97	CD97	TM7LN1
CD98	SLC3A2	4F2; 4F2HC; 4T2HC; CD98; MDU1; NACAE
CD99	CD99	MIC2; MIC2X; MIC2Y
CD99R	CD99	
CDW12	CDw12	CDw12; p90-120
CDw145	CDw145	not listed
CDw198	CCR8	CKR-L1; CKRL1; CMKBR8; CMKBRL2; CY6; GPR-CY6; TER1
CDw199	CCR9	GPR-9-6; GPR28
CDw210a	IL10RA	CDW210A; HIL-10R; IL-10R1; IL10R
CDw210b	IL10RB	CDW210B; CRF2-4; CRFB4; D21S58; D21S66; IL-10R2
CDw293	BMPR1B	BMPR1B; ALK6; ALK-6

Table 3: List of Membrane-Bound Receptors

Membrane-bound Receptor Name	mRNA ID
5-HT3 receptor subunit E splice variant HTR3Ea	DQ644022.1
5-HT3 serotonin receptor (long isoform)	AJ003078.1
5-HT3c1 serotonin receptor-like protein	AY349352.1 AY349353.1
5-hydroxytryptamine (serotonin) receptor 3 family member D	BC101091.2 BC101090.2 NM_001145143.1 NM_182537.2 AJ437318.1 AY159812.2 GI:110431739
5-hydroxytryptamine (serotonin) receptor 3, family member C (HTR3C)	NM_130770.2 BC131799.1 AF459285.1
5-hydroxytryptamine (serotonin) receptor 3, family member E (HTR3E)	NM_182589.2 BC101183.2 BC101185.2

Membrane-bound Receptor Name	mRNA ID
	BC101182.1 AY159813.2 EU165354.1
5-hydroxytryptamine (serotonin) receptor 3A (HTR3A)	BC004453.1 BC002354.2 BT007204.1 GI:30583246 NM_001161772.2 NM_213621.3 NM_000869.5 AF498984.1
5-hydroxytryptamine (serotonin) receptor 3B (HTR3B)	NM_006028.3 AK314268.1 AF169255.1 AF080582.1 AM293589.1
ABA-A receptor, alpha 1 subunit	X14766.1
ABC protein	AF146074.1
ABC transporter 7 protein	AB005289.1
ABC transporter MOAT-B (MOAT-B)	AF071202.1
ABC transporter MOAT-C (MOAT-C)	AF104942.1
ABC transporter MOAT-D (MOAT-D)	AF104943.1
ABC transporter umat (ABCB6 gene)	AJ289233.2
ABCB5 mRNA for ATP-binding cassette, sub-family B (MDR/TAP), member 5	AB353947.1
ABCC4 protein	AB208973.1
acetylcholine receptor (epsilon subunit)	X66403.1
acetylcholine receptor delta subunit	X55019.1 GI:297401
adrenoleukodystrophy related protein (ALDR)	AJ000327.1
ALD gene	Z21876.1
alpha 7 neuronal nicotinic acetylcholine receptor	U40583.1
alpha-1 strychnine binding subunit of inhibitory glycine receptor mRNA	X52009.1
alpha-2 strychnine binding subunit of inhibitory glycine receptor mRNA	X52008.1
alpha-3 neuronal nicotinic acetylcholine receptor subunit	M37981.1
amino butyric acid (GABA rho2) gene	M86868.1
amino butyric acid (GABAA) receptor beta-3 subunit	M82919.1
amma-aminobutyric acid (GABA) receptor, rho 1	BC130344.1
Anaplastic lymphoma receptor tyrosine kinase (ALK)	NM_004304.4
anthracycline resistance associated protein	X95715.1
ATP binding cassette transporter	AF038950.1
ATP-binding cassette (sub-family C, member 6) (ABCC6 gene)	AM774324.1 AM711638.1
ATP-binding cassette 7 iron transporter (ABC7)	AF133659.1
ATP-binding cassette C5	AB209103.1
ATP-binding cassette half-transporter (PRP)	AF308472.1
ATP-binding cassette protein (ABCB5)	AY230001.1 AY196484.1
ATP-binding cassette protein ABCB9 (ABCB9)	AF216494.1
ATP-binding cassette protein C11 (ABCC11)	AF367202.1 AF411579.1 AY040219.1 NM_003742.2

Membrane-bound Receptor Name	mRNA ID
ATP-binding cassette protein C12 (ABCC12)	AF395909.1 AF411578.1 AF411577.1 AF395908.1 AY040220.1
ATP-binding cassette protein C13	AY063514.1 AF518320.1
ATP-binding cassette protein M-ABC1	AF047690.1
ATP-binding cassette subfamily B member 5 (ABCB5)	AY785909.1 AY851365.1
ATP-binding cassette transporter C4 (ABCC4)	AY207008.1 AF541977.1
ATP-binding cassette transporter MRP8	AF352582.1
ATP-binding cassette, sub-family B (MDR/TAP), member 1 (ABCB1)	BC130424.1 NM_000927.4
ATP-binding cassette, sub-family B (MDR/TAP), member 10 (ABCB10)	BC064930.1 NM_012089.2 NM_001198934.1
ATP-binding cassette, sub-family B (MDR/TAP), member 4 (ABCB4)	BC042531.1 BC020618.2 NM_018849.2 NM_000443.3 NM_018850.2
ATP-binding cassette, sub-family B (MDR/TAP), member 5 (ABCB5)	BC104894.1 BC104920.1 NM_001163941.1 NM_178559.5
ATP-binding cassette, sub-family B (MDR/TAP), member 6 (ABCB6)	BC000559.2 NM_005689.2
ATP-binding cassette, sub-family B (MDR/TAP), member 7 (ABCB7)	BC006323.2 BT009918.1 NM_004299.3
ATP-binding cassette, sub-family B (MDR/TAP), member 8 (ABCB8)	BC151235.1 BC141836.1 BGI:146327013 NM_007188.3 AK222911.1
ATP-binding cassette, sub-family B (MDR/TAP), member 9 (ABCB9)	BC017348.2 BC064384.1 NM_019624.3 NM_019625.3 NM_203444.2
ATP-binding cassette, sub-family C (CFTR/MRP), member 1 (ABCC1)	NM_019898.2 NM_019899.2 NM_019862.2 NM_004996.3 NM_019900.2 AB209120.1
ATP-binding cassette, sub-family C (CFTR/MRP), member 10 (ABCC10)	NM_033450.2 GI:25914748
ATP-binding cassette, sub-family C (CFTR/MRP), member 11 (ABCC11)	NM_145186.2 NM_032583.3 NM_033151.3
ATP-binding cassette, sub-family C (CFTR/MRP), member 12 (ABCC12)	NM_033226.2

Membrane-bound Receptor Name	mRNA ID
ATP-binding cassette, sub-family C (CFTR/MRP), member 2 (ABCC2)	BC136419.1 GI:187953242 NM_000392.3
ATP-binding cassette, sub-family C (CFTR/MRP), member 3 (ABCC3)	BC046126.1 BC137347.1 BC137348.1 BC104952.1 BC050370.1 NM_001144070.1 NM_003786.3 AB208954.1
ATP-binding cassette, sub-family C (CFTR/MRP), member 4 (ABCC4)	BC041560.1 NM_001105515.1 NM_005845.3
ATP-binding cassette, sub-family C (CFTR/MRP), member 5 (ABCC5)	BC140771.1 NM_005688.2
ATP-binding cassette, sub-family C (CFTR/MRP), member 6 (ABCC6)	BC131732.1 NM_001171.5
ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8)	NM_000352.3
ATP-binding cassette, sub-family C (CFTR/MRP), member 9 (ABCC9)	NM_020298.2 NM_020297.2 NM_005691.2
ATP-binding cassette, sub-family D (ALD), member 1 (ABCD1)	BC025358.1 BC015541.1 NM_000033.3
ATP-binding cassette, sub-family D (ALD), member 2 (ABCD2)	BC104901.1 BC104903.1 NM_005164.3 AK314254.1
ATP-binding cassette, sub-family D (ALD), member 3 (ABCD3)	BC009712.2 BC068509.1 BT006644.1 NM_001122674.1 NM_002858.3
ATP-binding cassette, sub-family D (ALD), member 4 (ABCD4)	BC012815.2 BT007412.1 NM_005050.3
beta 4 nicotinic acetylcholine receptor subunit	U48861.1
bile salt export pump (BSEP)	AF136523.1 AF091582.1
B-lymphocyte CR2-receptor (for complement factor C3d and Epstein-Barr virus)	Y00649.1
Butyrophilin-like 2 (MHC class II associated) (BTNL2)	NM_019602.1
Cadherin 1, type 1, E-cadherin (epithelial) (CDH1)	NM_004360.3
Cadherin 13, H-cadherin (heart) (CDH13)	NM_001257.3
Cadherin 15, type 1, M-cadherin (myotubule) (CDH15)	NM_004933.2
Cadherin 16, KSP-cadherin (CDH16)	NM_001204746.1 NM_001204745.1 NM_001204744.1 NM_004062.3
Cadherin 17, LI cadherin (liver-intestine) (CDH17)	NM_001144663.1 NM_004063.3
Cadherin 19, type 2 (CDH19)	NM_021153.2
Cadherin 2, type 1, N-cadherin (neuronal) (CDH2)	NM_001792.3
cadherin 20, type 2 (CDH20)	NM_031891.2
Cadherin 3, type 1, P-cadherin (CDH3)	NM_001793.4

Membrane-bound Receptor Name	mRNA ID
Cadherin 4, type 1, R-cadherin (CDH4)	NM_001794.2
Cadherin 5, type 2 (CDH5)	NM_001795.3
Cadherin 6, type 2, K-cadherin (CDH6)	NM_004932.2
Cadherin 7, type 2 (CDH7)	NM_004361.2 NM_033646.1
canalicular multidrug resistance protein	X96395.2
canalicular multispecific organic anion transporter (cMOAT)	U63970.1 U49248.1
Ccanalicular multispecific organic anion transporter 2 (CMOAT2)	AF083552.1
CD163 molecule-like 1 (CD163L1)	NM_174941.4
CD4 molecule (CD4)	NM_001195015.1 NM_001195017.1 NM_001195016.1 NM_001195014.1 NM_000616.4
CD47 molecule	BC010016.2 BT006907.1 BC037306.1 BC012884.1 NM_198793.2 NM_001777.3
cellular proto-oncogene (c-mer)	U08023.1
ceptor for advanced glycosylation end-products intron 4&9 variant (AGER)	AY755622.1
Cholinergic receptor, nicotinic, alpha 1 (CHRNA1)	NM_000079.3 NM_001039523.2 AK315312.1
Cholinergic receptor, nicotinic, alpha 10 (CHRNA10)	NM_020402.2
Cholinergic receptor, nicotinic, alpha 2 (CHRNA2)	BC153866.1 NM_000742.3
Cholinergic receptor, nicotinic, alpha 3 (CHRNA3)	BC002996.1 BC098443.1 BC000513.2 BC001642.2 BC006114.1 NM_001166694.1 NM_000743.4 BT006897.1 BT006646.1
Cholinergic receptor, nicotinic, alpha 4 (CHRNA4)	BC096293.3 GI:109731542 BC096290.1 BC096292.1 BC096291.1 NM_000744.5 AB209359.1
Cholinergic receptor, nicotinic, alpha 5 (CHRNA5)	BC033639.1 NM_000745.3
Cholinergic receptor, nicotinic, alpha 6 (CHRNA6)	BC014456.1 NM_001199279.1 NM_004198.3 AK313521.1
Cholinergic receptor, nicotinic, alpha 7 (CHRNA7)	BC037571.1 NM_000746.4 NM_001190455.1
Cholinergic receptor, nicotinic, alpha 9 (CHRNA9)	BC113549.1 BC113575.1 NM_017581.2

Membrane-bound Receptor Name	mRNA ID
Cholinergic receptor, nicotinic, beta 1 (CHRNB1)	BC023553.2 BC011371.1 NM_000747.2
Cholinergic receptor, nicotinic, beta 2 (CHRNB2)	BC075041.2 BC075040.2 AK313470.1 NM_000748.2
Cholinergic receptor, nicotinic, beta 3 (CHRNB3)	BC069788.1 BC069703.1 BC069681.1 NM_000749.3
Cholinergic receptor, nicotinic, beta 4 (CHRNB4)	BC096080.1 BC096082.1 NM_000750.3
cholinergic receptor, nicotinic, delta (CHRND)	BC093925.1 BC093923.1 NM_000751.1
Cholinergic receptor, nicotinic, epsilon (CHRNE)	NM_000080.3
Cholinergic receptor, nicotinic, gamma (CHRNG)	BC111802.1 NM_005199.4
CRB1 isoform II precursor	AY043325.1
Cstic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7) (CFTR)	NM_000492.3
C-type lectin domain family 4, member A (CLEC4A)	NM_194450.2 NM_194448.2 NM_194447.2 NM_016184.3
enaptin	AF535142.1
Eph-related receptor transmembrane ligand Elk-L3 precursor (Elk-L3)	U62775.1
Fc receptor related gene	DQ021957.1
Fibroblast growth factor receptor 3 (FGFR3)	NM_022965.3
Fibroblast growth factor receptor 4 (FGFR4)	NM_022963.2
Fms-related tyrosine kinase 3 (FLT3)	NM_004119.2
Follicle stimulating hormone receptor (FSHR)	AY429104.1 S59900.1 M95489.1 M65085.1 BC118548.1 BC096831.1 BC125270.1 NM_181446.2 NM_000145.3 X68044.1
G protein-coupled receptor 155 (GPR155)	BC035037.1 BC028730.1 NM_001033045.2 NM_152529.5
GABA-A receptor delta subunit (GABRD)	AF016917.1
GABA-A receptor epsilon subunit	U66661.1
GABAA receptor gamma 3 subunit	S82769.1
GABA-A receptor pi subunit	U95367.1
GABAA receptor subunit alpha4	U30461.1
GABA-A receptor theta subunit (THETA)	AF189259.1 AF144648.1
GABA-A receptor, beta 1 subunit	X14767.1

Membrane-bound Receptor Name	mRNA ID
GABA-A receptor, gamma 2 subunit	X15376.1
GABA-benzodiazepine receptor alpha-5-subunit (GABRA5)	L08485.1
Gamma-aminobutyric acid (GABA) A receptor, alpha 1 (GABRA1)	BC030696.1 NM_001127648.1 NM_001127647.1 NM_001127646.1 NM_001127645.1 NM_001127644.1 NM_001127643.1 NM_000806.5
Gamma-aminobutyric acid (GABA) A receptor, alpha 2 (GABRA2)	BC022488.1 NM_001114175.1 NM_000807.2
Gamma-aminobutyric acid (GABA) A receptor, alpha 3 (GABRA3)	BC028629.1 NM_000808.3
Gamma-aminobutyric acid (GABA) A receptor, alpha 4 (GABRA4)	BC035055.1 NM_001204267.1 NM_001204266.1 NM_000809.3
Gamma-aminobutyric acid (GABA) A receptor, alpha 5 (GABRA5)	BC113422.1 BC111979.1 BT009830.1 NM_001165037.1 NM_000810.3
Gamma-aminobutyric acid (GABA) A receptor, alpha 6 (GABRA6)	BC099641.3 BC096241.3 BC099640.3 BC096242.3 NM_000811.2
Gamma-aminobutyric acid (GABA) A receptor, beta 1 (GABRB1)	BC022449.1 NM_000812.3
Gamma-aminobutyric acid (GABA) A receptor, beta 2 (GABRB2)	BC105639.1 BC099719.1 BC099705.1 NM_021911.2 NM_000813.2
gamma-aminobutyric acid (GABA) A receptor, beta 3 (GABRB3)	BC010641.1 NM_001191320.1 NM_021912.4 NM_001191321.1 NM_000814.5
Gamma-aminobutyric acid (GABA) A receptor, delta (GABRD)	BC033801.1 NM_000815.4
Gamma-aminobutyric acid (GABA) A receptor, epsilon (GABRE)	BC059376.1 BC047108.1 BC026337.1 NM_004961.3
Gamma-aminobutyric acid (GABA) A receptor, gamma 1 (GABRG1)	BC031087.1 NM_173536.3

Membrane-bound Receptor Name	mRNA ID
Gamma-aminobutyric acid (GABA) A receptor, gamma 2 (GABRG2)	BC074795.2 GI:50959646 BC059389.1 NM_198903.2 NM_000816.3 NM_198904.2
Gamma-aminobutyric acid (GABA) A receptor, gamma 3 (GABRG3)	NM_033223.4
Gamma-aminobutyric acid (GABA) A receptor, pi (GABRP)	BC074810.2 BC069348.1 BC074865.2 BC109105.1 BC109106.1 NM_014211.2
Gamma-aminobutyric acid (GABA) receptor, rho 1 (GABRR1)	NM_002042.3
Gamma-aminobutyric acid (GABA) receptor, rho 2 (GABRR2)	BC130352.1 BC130354.1 NM_002043.2
gamma-aminobutyric acid (GABA) receptor, rho 3 (GABRR3)	NM_001105580.1
gamma-aminobutyric acid (GABA) receptor, theta (GABRQ)	BC109210.1 BC109211.1 NM_018558.2
gamma-aminobutyric acid A receptor beta 2 isoform 3 (GABRB2)	GU086164.1 GU086163.1
gamma-aminobutyric acid A receptor beta 2 subunit (GABR2)	S67368.1
gamma-aminobutyric acid A receptor, alpha 2 precursor	AB209295.1
gamma-aminobutyric acid receptor type A rho-1 subunit (GABA-A rho-1)	M62400.1
gamma-aminobutyric acid type A receptor alpha 6 subunit	S81944.1
gamma-aminobutyric acidA receptor alpha 2 subunit	S62907.1
gamma-aminobutyric acidA receptor alpha 3 subunit	S62908.1
gamma-aminobutyric-acid receptor alpha-subunit	X13584.1
glycine receptor alpha 3 subunit	U93917.1
glycine receptor alpha2 subunit B (GLRA2)	AY437084.1 AY437083.1
glycine receptor beta subunit precursor (GLRB)	AF094755.1 AF094754.1
Glycine receptor, alpha 1 (GLRA1)	BC114967.1 BC114947.1 BC074980.2 NM_001146040.1 NM_000171.3
Glycine receptor, alpha 2 (GLRA2)	BC032864.2 NM_001171942.1 NM_001118886.1 NM_001118885.1 NM_002063.3
Glycine receptor, alpha 3 (GLRA3)	BC036086.1 NM_006529.2 NM_001042543.1
Glycine receptor, alpha 4 (GLRA4)	NM_001172285.1 NM_001024452.2
glycine receptor, beta (GLRB)	BC032635.1 NM_001166061.1 NM_000824.4 NM_001166060.1

Membrane-bound Receptor Name	mRNA ID
GP2	D38225.1
gpVI mRNA for platelet glycoprotein VI	AB035073.1
H1 histamine receptor	Z34897.1
HEK2 protein tyrosine kinase receptor	X75208.1
high affinity IgE receptor alpha-subunit (FcERI)	X06948.1
HLA	D32131.1 D32129.1
IILA class I locus C heavy chain	X58536.1
HLA class II DR-beta (HLA-DR B)	X12544.1
HLA classII histocompatibility antigen alpha-chain	X00452.1
HLA-A26 (HLA class-1 heavy chain)	D32130.1
HLA-DR antigens associated invariant chain (p33)	X00497.1
holinergic receptor, nicotinic, delta polypeptide(CHRND)	AK315297.1
HPTP (protein tyrosine phosphatase delta)	X54133.1
HPTP (protein tyrosine phosphatase epsilon)	X54134.1
HPTP (protein tyrosine phosphatase zeta)	X54135.1
HPTP alpha mRNA for protein tyrosine phosphatase alpha	X54130.1
HPTP beta (protein tyrosine phosphatase beta)	X54131.1
-hydroxytryptamine (serotonin) receptor 3 family member D (HTR3D)	NM_001163646.1
ICAM-3	X69819.1
IL12 receptor component	U03187.1
IL-4-R	X52425.1
immunoglobulin receptor precursor	AY046418.1
insulin-like growth factor I receptor	X04434.1
integrin associated protein	Z25521.1
Killer cell lectin-like receptor subfamily D, member 1 (KLRD1)	NM_001114396.1
KIR (cl-11) NK receptor precursor protein	U30274.1 U30273.1 U30272.1
large conductance calcium- and voltage-dependent potassium channel alpha subunit (MaxiK)	U11058.2
large-conductance calcium-activated potassium channel beta subunit (KCNMB4)	AF160967.1
leucine-rich glioma-inactivated protein precursor (LGI1)	AF055636.1
Leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2 (LILRA2)	NM_001130917.1 NM_006866.2
Leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 3 (LILRA3)	NM_006865.3 NM_001172654.1
lycine receptor beta subunit (GLRB)	U33267.1
lymphocte activation marker Blast-1	X06341.1
M-ABC2 protein (M-ABC2), nuclear gene for mitochondrial product	AF216833.1
Major histocompatibility complex, class I, A (HLA-A)	NM_002116.6
Major histocompatibility complex, class I, B (HLA-B)	NM_005514.6
Major histocompatibility complex, class I, C (HLA-C)	NM_002117.4
Major histocompatibility complex, class I, E (HLA-E)	NM_005516.5
Major histocompatibility complex, class I, G (HLA-G),	NM_002127.5
MAT8 protein	X93036.1
MCTP1L mRNA	AY656715.1
MCTP1S	AY656716.1
MCTP2	AY656717.1
membrane glycoprotein P (mdr3)	M23234.1
Mint1	AF029106.1
mono ATP-binding cassette protein	AB013380.1 GI:12248754
MRP5	AB019002.1
MRP6 (MRP6)	AF076622.1
MT-ABC transporter (MTABC)	AF076775.1

Membrane-bound Receptor Name	mRNA ID
multidrug resistance protein 1	EU854148.1 EU852583.1 AB208970.1
multidrug resistance protein 3 (ABCC3)	Y17151.2
multidrug resistance protein 5 (MRP5)	U83661.2
multidrug resistance-associated protein (ABCC4)	AY081219.1
multidrug resistance-associated protein (MRP)	L05628.1
multidrug resistance-associated protein 3 (MRP3)	AF085690.1 AF085691.1
Multidrug resistance-associated protein 5 variant protein	AB209454.1
multidrug resistance-associated protein 7 (SIMRP7)	AY032599.1
multidrug resistance-associated protein homolog MRP3 (MRP3)	AF009670.1
multidrug resistance-associated protein(MRP)-like protein-2 (MLP-2)	AB010887.1
multiple C2 domains, transmembrane 1 (MCTP1)	BC030005.2 NM_001002796.2 NM_024717.4
multiple C2 domains, transmembrane 2 (MCTP2)	BC111024.1 BC041387.1 BC025708.1 BC131527.1 NM_001159644.1 NM_018349.3 NM_001159643.1
myeloid cell leukemia ES variant (MCL1)	FJ917536.1
neuregulin 4 (NRG4)	AM392365.1 AM392366.1
neuronal nAChR beta-3 subunit	X67513.1
neuronal nicotinic acetylcholine alpha10 subunit (NACHRA10 gene)	AJ278118.1 AJ295237.1
neuronal nicotinic acetylcholine receptor alpha-3 subunit	X53559.1
nicotinic acetylcholine alpha-7 subunit (CHRNA7 gene)	X70297.1 AJ586911.1
neuronal nicotinic acetylcholine receptor beta-2 subunit	X53179.1
nicotinic acetylcholine receptor alpha 3 subunit precursor	M86383.1
nicotinic acetylcholine receptor alpha 4 subunit (nAChR)	L35901.1
nicotinic acetylcholine receptor alpha 9 subunit (NACHRA9 gene)	AJ243342.1
nicotinic acetylcholine receptor alpha2 subunit precursor	U62431.1 Y16281.1
nicotinic acetylcholine receptor alpha3 subunit precursor	U62432.1 Y08418.1
nicotinic acetylcholine receptor alpha4 subunit precursor	U62433.1 Y08421.1 X87629.1
nicotinic acetylcholine receptor alpha5 subunit precursor	U62434.1 Y08419.1
nicotinic acetylcholine receptor alpha6 subunit precursor	U62435.1 Y16282.1
nicotinic acetylcholine receptor alpha7 subunit precursor	U62436.1
nicotinic acetylcholine receptor alpha7 subunit precursor	Y08420.1
nicotinic acetylcholine receptor beta2 subunit precursor	U62437.1
nicotinic acetylcholine receptor beta2 subunit precursor	Y08415.1
nicotinic acetylcholine receptor beta3 subunit precursor	U62438.1
nicotinic acetylcholine receptor beta3 subunit precursor	Y08417.1
nicotinic acetylcholine receptor beta4 subunit precursor	U62439.1
nicotinic acetylcholine receptor beta4 subunit precursor	Y08416.1

Membrane-bound Receptor Name	mRNA ID
nicotinic acetylcholine receptor subunit alpha 10	AF199235.2
nicotinic cholinergic receptor alpha 7 (CHRNA7)	AF385585.1
nicotinic receptor alpha 5 subunit	M83712.1
nicotinic receptor beta 4 subunit	X68275.1
on-erythroid band 3-like protein (HKB3)	X03918.1
p58 natural killer cell receptor precursor	U24079.1 U24078.1 U24077.1 U24076.1 U24075.1 U24074.1
peptide transporter (TAP1)	L21207.1 L21206.1 L21205.1 L21204.1
peroxisomal 70 kD membrane protein	M81182.1
peroxisomal membrane protein 69 (PMP69)	AF009746.1
P-glycoprotein	AY090613.1
P-glycoprotein (ABCB1)	AF399931.1 AF319622.1
P-glycoprotein (mdr1)	AF016535.1
P-glycoprotein (PGY1)	M14758.1
P-glycoprotein ABCB5	AY234788.1
Phospholipase A2 receptor 1, 180kDa (PLA2R1)	NM_001007267.2
PMP70	X58528.1
Potassium voltage-gated channel, shaker-related subfamily, member 5 (KCNA5)	NM_002234.2
potassium voltage-gated channel, shaker-related subfamily, member 7 (KCNA7)	NM_031886.2
precursor of epidermal growth factor receptor	X00588.1
pre-T cell receptor alpha-type chain precursor	U36759.1
protein tyrosine phosphatase hPTP-J precursor	U73727.1
Protein tyrosine phosphatase, receptor type, F (PTPRF) 2	NM_006504.4 NM_130435.3 NM_002840.3 NM_130440.2
Protein tyrosine phosphatase, receptor type, G (PTPRG)	NM_002841.3
Protein tyrosine phosphatase, receptor type, H (PTPRH)	NM_001161440.1 NM_002842.3
Protein tyrosine phosphatase, receptor type, J (PTPRJ)	NM_002843.3 NM_001098503.1
Protein tyrosine phosphatase, receptor type, K (PTPRK)	NM_001135648.1 NM_002844.3
Protein tyrosine phosphatase, receptor type, M (PTPRM)	NM_001105244.1 NM_002845.3
Protein tyrosine phosphatase, receptor type, N polypeptide 2 (PTPRN2)	NM_001199764.1 NM_002846.3 NM_001199763.1 NM_130843.2 NM_002847.3 NM_130842.2
Protein tyrosine phosphatase, receptor type, R (PTPRR)	NM_130846.1 NM_002849.2
Protein tyrosine phosphatase, receptor type, T (PTPRT)	NM_007050.5 NM_133170.3
Protein tyrosine phosphatase, receptor type, U (PTPRU)	NM_001195001.1 NM_133178.3
protein tyrosine phosphatase, receptor type, U (PTPRU)	NM_005704.4 NM_133177.3
protocadherin 1 (PCDH1)	NM_002587.3 NM_032420.2
Protocadherin 8 (PCDH8), transcript variant 2	NM_032949.2 NM_002590.3
Protocadherin 9 (PCDH9)	NM_203487.2 NM_020403.4

Membrane-bound Receptor Name	mRNA ID
protocadherin alpha 1 (PCDHA1)	NM_031411.1
Protocadherin alpha 10 (PCDHA10)	NM_031860.1
protocadherin alpha 6 (PCDHA6)	NM_031849.1
protocadherin gamma subfamily A, 1 (PCDHGA1)	NM_018912.2 NM_031993.1
protocadherin gamma subfamily A, 10 (PCDHGA10)	NM_018913.2 NM_032090.1
Protocadherin gamma subfamily A, 11 (PCDHGA11)	NM_032092.1 NM_032091.1 NM_018914.2
Protocadherin gamma subfamily A, 12 (PCDHGA12)	NM_032094.1 NM_003735.2
Protocadherin gamma subfamily A, 2 (PCDHGA2)	NM_032009.1 NM_018915.2
protocadherin gamma subfamily A, 3 (PCDHGA3)	NM_018916.3
protocadherin gamma subfamily A, 3 (PCDHGA3)	NM_032011.1
protocadherin gamma subfamily A, 4 (PCDHGA4)	NM_032053.1 NM_018917.2
protocadherin gamma subfamily A, 5 (PCDHGA5)	NM_032054.1 NM_018918.2
protocadherin gamma subfamily A, 6 (PCDHGA6), transcript variant 2	NM_032086.1 NM_018919.2
protocadherin gamma subfamily A, 7 (PCDHGA7)	NM_018920.2 NM_032087.1
Protocadherin gamma subfamily A, 8 (PCDHGA8)	NM_032088.1 NM_014004.2
protocadherin gamma subfamily A, 9 (PCDHGA9)	NM_018921.2 NM_032089.1
protocadherin gamma subfamily B, 1 (PCDHGB1)	NM_018922.2 NM_032095.1
protocadherin gamma subfamily B, 2 (PCDHGB2)	NM_018923.2 NM_032096.1
protocadherin gamma subfamily B, 3 (PCDHGB3)	NM_018924.2 NM_032097.1
Protocadherin gamma subfamily B, 4 (PCDHGB4)	NM_032098.1 NM_003736.2
protocadherin gamma subfamily B, 5 (PCDHGB5)	NM_032099.1 NM_018925.2
protocadherin gamma subfamily B, 6 (PCDHGB6)	NM_032100.1 NM_018926.2
Protocadherin gamma subfamily B, 7 (PCDHGB7)	NM_032101.1 NM_018927.2
Protocadherin gamma subfamily C, 3 (PCDHGC3)	NM_032403.1 NM_032402.1 NM_002588.2
protocadherin gamma subfamily C, 4 (PCDHGC4)	NM_018928.2 NM_032406.1
protocadherin gamma subfamily C, 5 (PCDHGC5)	NM_032407.1 NM_018929.2
PSF-2	M74447.1
transmembrane receptor IL-1Rrp	U43672.1
RING4	X57522.1
Sarcoglycan, zeta (SGCZ)	NM_139167.2
SB classII histocompatibility antigen alpha-chain	X00457.1
SH2 domain-containing phosphatase anchor protein 1c (SPAP1)	AF319440.1
SMRP	AB005659.1
Solute carrier family 4, sodium bicarbonate cotransporter, member 4 (SLC4A4)	NM_001134742.1 NM_003759.3 NM_001098484.2
Solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2 (SLC6A2)	NM_001172504.1 NM_001172502.1 NM_001172501.1 NM_001043.3
sulfonylurea receptor (SUR1)	U63421.1 AB209084.1 AF087138.1
sushi-repeat-containing protein precursor (SRPX)	U78093.1

Membrane-bound Receptor Name	mRNA ID
Synaptotagmin XIII (SYT13)	NM_020826.2
Synaptotagmin XV (SYT15)	NM_031912.4 NM_181519.2
T200 leukocyte common antigen (CD45, LC-A)	Y00062.1
TAP2B	Z22935.1
TAP2E	Z22936.1
TAPL (TAP-Like),	AB112583.1 AB112582.1 AB045381.2
thyroperoxidase	Y00406.1
tissue-type tonsil IFGP6	AY212514.1
trans-golgi network glycoprotein 48 (TGN)	AF027515.1
trans-golgi network glycoprotein 51 (TGN)	AF027516.1
Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) (TAP1)	BC014081.2 NM_000593.5 AY523971.2 AY523970.1
Transporter 2, ATP-binding cassette, sub-family B (MDR/TAP) (TAP2),	AF078671.1 AF105151.1 NM_018833.2 NM_000544.3 AK223300.1 AK222823.1 AB073779.1
	AB208953.1
ATP-binding cassette transporter sub-family C member 13 (ABCC13)	AY344117.1
tyrosine kinase (FER)	J03358.1
Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1 (UQCRFS1)	NM_006003.2 BC067832.1 BC010035.2 BC000649.1
ulfonylurea receptor (SUR1)	L78207.1

Cell-type specific polypeptides

[00458] As used herein, the term "cell-type specific polypeptide" refers to a polypeptide that is expressed in a cell having a particular phenotype (*e.g.*, a muscle cell) but is not generally expressed in other cell types with different phenotypes. For example, MyoD is expressed specifically in muscle cells but not in non-muscle cells, thus MyoD is a cell-type specific polypeptide. As another example, albumin is expressed in hepatocytes and is thus an hepatocyte-specific polypeptide.

[00459] Such cell-specific polypeptides are well known in the art or can be found using a gene array analysis and comparison of at least two different cell types. Methods for gene expressional array analysis is well known in the art.

[00460] Differentiation factors, reprogramming factors and transdifferentiation factors are further discussed herein in their appropriate sub-sections.

Death Receptors and Death Receptor Ligands

[00461] By "death receptor" is meant a receptor that induces cellular apoptosis once bound by a ligand. Death receptors include, for example, tumor necrosis factor (TNF) receptor superfamily members having death domains (*e.g.*, TNFRI, Fas, DR3, 4, 5, 6) and TNF receptor superfamily

members without death domains LTbetaR, CD40, CD27, HVEM. Death receptors and death receptor ligands are well known in the art or are discussed herein.

[00462] The synthetic, modified RNAs described herein can encode for death receptors to be expressed on the surface of a cell to enhance the vulnerability of a cell to apoptosis. The death ligand can also be encoded or can be provided *e.g.*, at a tumor site. This is particularly useful in the treatment of cancer, where cells evade apoptosis and continue to divide. Alternatively, the synthetic, modified RNAs or compositions thereof can encode for a death receptor ligand, which will induce apoptosis in cells that express a cell surface death receptor and can increase the efficiency of programmed cell death in targeted cells of a subject.

[00463] Some non-limiting examples of death receptors include FAS (CD95, Apo1), TNFR1 (p55, CD120a), DR3 (Apo3, WSL-1, TRAMP, LARD), DR4, DR5 (Apo2, TRAIL-R2, TRICK2, KILLER), CAR1, and the adaptor molecules FADD, TRADD, and DAXX. Some non-limiting examples of death receptor ligands include FASL (CD95L), TNF, lymphotoxin alpha, Apo3L (TWEAK), and TRAIL (Apo2L).

Mitogen Receptors

[00464] The synthetic, modified RNAs described herein can be used to express a mitogen receptor on a cell surface. Activation of a mitogen receptor with the mitogen induces cell growth and/or differentiation of the cell.

[00465] Mitogen receptors include those that bind ligands including, but not limited to: insulin, insulin-like growth factor (*e.g.*, IGF1, IGF2), platelet derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), fibroblast growth factor (FGF), bone morphogenic proteins (BMPs), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), transforming growth factor (TGF)-alpha and -beta, among others.

[00466] In addition, cytokines that promote cell growth can also be encoded by synthetic, modified RNAs herein. For example, cytokines such as erythropoietin, thrombopoietin and other cytokines from the IL-2 sub-family tend to induce cell proliferation and growth.

Protein Therapeutics

[00467] Synthetic, modified RNAs as described herein can also be used to express protein therapeutically in cells by either administration of a synthetic, modified RNA composition to an individual or by administering a synthetic, modified RNA to cells that are then introduced to an individual. In one aspect, cells can be transfected with a modified RNA to express a therapeutic protein using an *ex vivo* approach in which cells are removed from a patient, transfected by *e.g.*, electroporation or lipofection, and re-introduced to the patient. Continuous or prolonged administration in this manner can be achieved by electroporation of blood cells that are re-infused to the patient.

[00468] Some exemplary protein therapeutics include, but are not limited to: insulin, growth hormone, erythropoietin, granulocyte colony-stimulating factor (G-CSF), thrombopoietin, clotting factor VII, Factor IX, interferon, glucocerebrosidase, anti-HER2 monoclonal antibody, and Etanercept, among others.

[00469] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Thus for example, references to "the method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth. In addition, the term 'cell' can be construed as a cell population, which can be either heterogeneous or homogeneous in nature, and can also refer to an aggregate of cells.

[00470] It is understood that the foregoing detailed description and the following examples are illustrative only and are not to be taken as limitations upon the scope of the invention. Various changes and modifications to the disclosed embodiments, which will be apparent to those of skill in the art, may be made without departing from the spirit and scope of the present invention. Further, all patents, patent applications, and publications identified are expressly for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents are based on the information available to the applicants and do not constitute any admission as to the correctness of the dates or contents of these documents.

[00471]

EXAMPLES

[00472] Currently, clinical applications using induced pluripotent stem (iPS) cells are impeded by low efficiency of iPS derivation, and the use of protocols that permanently modify the genome to effect cellular reprogramming. Moreover, safe, reliable, and effective means of directing the fate of patient-specific iPS cells towards clinically useful cell types are lacking. Described herein are novel, non-mutagenic strategies for altering cellular phenotypes, such as reprogramming cell fate, based on the administration of synthetic, modified mRNAs that are modified to overcome innate cellular anti-viral responses. The compositions and approaches described herein can be used to reprogram multiple human cell types to pluripotency with surprising and unexpected efficiencies that

greatly surpass established protocols. Also described herein are novel compositions and methods for directing the fate of cells towards clinically useful cell types, and a non-limiting example that demonstrates that this technology can be used to efficiently direct the differentiation of RNA-induced pluripotent stem (RiPS) cells into terminally differentiated myogenic cells. Thus, the compositions and methods described herein represent safe, highly efficient strategies for altering cellular developmental potentials, such as somatic cell reprogramming and directing differentiated cell fates, that have broad applicability for basic research, disease modeling and regenerative and personalized medicine.

Experimental Procedures

Construction of IVT templates

[00473] The pipeline for production of IVT template constructs and subsequent RNA synthesis is schematized in Figure 1. The oligonucleotide sequences used in the construction of IVT templates are shown in Table 4. All oligos were synthesized by Integrated DNA Technologies (Coralville, IA). ORF PCRs were templated from plasmids bearing human KLF4, c-MYC, OCT4, SOX2, human ES cDNA (LIN28), Clontech pIRES-eGFP (eGFP), pRVGP (d2eGFP) and CMV-MyoD from Addgene. The ORF of the low-stability nuclear GFP was constructed by combining the d2eGFP ORF with a 3' nuclear localization sequence. PCR reactions were performed using HiFi Hotstart™ (KAPA Biosystems, Woburn, MA) per the manufacturer's instructions. Splint-mediated ligations were carried out using Ampligase™ Thermostable DNA Ligase (Epicenter Biotechnologies, Madison, WI). UTR ligations were conducted in the presence of 200 nM UTR oligos and 100 nM splint oligos, using 5 cycles of the following annealing profile: 95°C for 10 seconds; 45°C for 1 minute; 50°C for 1 minute; 55°C for 1 minute; 60°C for 1 minute. A phosphorylated forward primer was employed in the ORF PCRs to facilitate ligation of the top strand to the 5' UTR fragment. The 3' UTR fragment was also 5'-phosphorylated using polynucleotide kinase (New England Biolabs, Ipswich, MA). All intermediate PCR and ligation products were purified using QIAquick™ spin columns (Qiagen, Valencia, CA) before further processing. Template PCR amplicons were sub-cloned using the pcDNA 3.3-TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Plasmid inserts were excised by restriction digest and recovered with SizeSelect gels (Invitrogen) before being used to template tail PCRs.

[00474] 5' and 3' UTR oligos are ligated to the top strand of gene-specific ORF amplicons to produce a basic template construct for cloning. Underlined bases in the 5' UTR oligo sequence indicate the upstream T7 promoter, and in the 3' UTR oligo sequence show downstream restriction sites, introduced to facilitate linearization of template plasmids. Template PCR primers are used to amplify ligation products for sub-cloning. Tail PCR primers are used to append an oligo(dT) sequence immediately after the 3' UTR to drive templated addition of a poly(A) tail during IVT reactions. Gene-specific ORF primers are used to capture the coding region (minus the start codon) from cDNA templates. Splint oligos mediate ligation of UTR oligos to the top strand of ORF amplicons.

Table 4: Oligonucleotides for IVT template construction (SEQ ID NOs: 1429-1466, respectively, in order of appearance)

	ORF Forward Primer	ORF Reverse Primer
eGFP	GTGAGCAAGGGCGAGGAGCTGTT	TTACTTGTACAGCTCGTCCATGCCGAGA
d2eGFP	GTGAGCAAGGGCGAGGAGCTGTT	CTACACATTGATCCTAGCAGAAGCACAGGCT
KLF4	GCTGTCAGCGACGCGCTGCTC	TTAAAAATGCCTCTTCATGTGTAAGGCGAGGT
c-MYC	CCCCTCAACGTTAGCTTCACCAACAGG	TTACGCACAAGAGTTCCGTAGCTGTTCA
OCT4	GCGGGACACCTGGCTTCGGATTTC	TCAGTTTGAATGCATGGGAGAGCCCAGA
SOX2	TACAACATGATGGAGACGGAGCTGAAGC	TCACATGTGTGAGAGGGGCAGTGTG
LIN28	GGCTCCGTGTCCAACCAG	TCAATTCTGTGCCTCCGG
MYOD	GAGCTTCTATCGCCGCCACTCC	TCAAAGCACCTGATAAATCGCATTGG
	5' Splint Oligo	**3' Splint Oligo**
eGFP	TCCTCGCCCTTGCTCACCATGGTGGCTCTTATATTTCTTCTT	CCCGCAGAAGGCAGCTTACTTGTACAGCTCGTCCATGC
d2eGFP	TCCTCGCCCTTGCTCACCATGGTGGCTCTTATATTTCTTCTT	CCCGCAGAAGGCAGCCTACACATTGATCCTAGCAGA
KLF4	GCGCGTCGCTGACAGCCATGGTGGCTCTTATATTTCTTCTT	CCCGCAGAAGGCAGCTTAAAAATGCCTCTTCATGTGTAA
c-MYC	GTGAAGCTAACGTTGAGGGGCATGGTGGCTCTTATATTTCTTCTT	CCCGCAGAAGGCAGCTTACGCACAAGAGTTCCGTAG
OCT4	AAGCCAGGTGTCCCGCCATGGTGGCTCTTATATTTCTTCTT	CCCGCAGAAGGCAGCTCAGTTTGAATGCATGGGAG
SOX2	CTCCGTCTCCATCATGTTGTACATGGTGGCTCTTATATTTCTTCTT	CCCGCAGAAGGCAGCTCACATGTGTGAGAGGGGC
LIN28	CTGGTTGGACACGGAGCCCATGGTGGCTCTTATATTTCTTCTT	CCCGCAGAAGGCAGCTCAATTCTGTGCCTCCGG
MYOD	TGGCGGCGATAGAAGCTCCATGGTGGCTCTTATATTTCTTCTT	CCCGCAGAAGGCAGCTCAAAGCACCTGATAAATCGCATTGG
	UTR Oligos	
5' UTR	TTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATG	
3' UTR	GCTGCCTTCTGCGGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGTGAGGGTCTAGAACTAGTGTCGACGC	
	Forward Primer	**Reverse Primer**
Template PCR	TTGGACCCTCGTACAGAAGCTAATACG	GCGTCGACACTAGTTCTAGACCCTCA
Tail PCR	TTGGACCCTCGTACAGAAGCTAATACG	T_{120}CTTCCTACTCAGGCTTTATTCAAAGACCA

Synthesis of synthetic, modified RNA

[00475] RNA was synthesized with the MEGAscript™ T7 kit (Ambion, Austin, TX), using 1.6 ug of purified tail PCR product to template each 40 uL reaction. A custom ribonucleoside blend was used comprising 3´-0-Me-m7G(5')ppp(5')G ARCA cap analog (New England Biolabs), adenosine triphosphate and guanosine triphosphate (USB, Cleveland, OH), 5-methylcytidine triphosphate and pseudouridine triphosphate (TriLink Biotechnologies, San Diego, CA). Final nucleotide reaction concentrations were 33.3 mM for the cap analog, 3.8 mM for guanosine triphosphate, and 18.8 mM for the other nucleotides. Reactions were incubated 3-6 hours at 37°C and DNAse-treated as directed by the manufacturer. RNA was purified using Ambion MEGAclear spin columns, then treated with Antarctic Phosphatase (New England Biolabs) for 30 minutes at 37°C to remove residual 5'-triphosphates. Treated RNA was re-purified, quantitated by Nanodrop™ (Thermo Scientific, Waltham, MA), and adjusted to 100 ng/uL working concentration by addition of Tris-EDTA (pH 7.0). RNA reprogramming cocktails were prepared by pooling individual 100 ng/uL RNA stocks to produce a 100 ng/uL (total) blend. The KMOS[L]+GFP cocktails were formulated to give equal molarity for each component except for OCT4, which was included at 3x molar concentration. Volumetric ratios used for pooling were as follows: 170:160:420:130:120[:90] (KLF4:c-MYC:OCT4:SOX2:GFP[:LIN28]).

Cells

[00476] The following primary cells were obtained from ATCC (Manassas, VA): human neonatal epidermal keratinocytes, BJ human neonatal foreskin fibroblasts, MRC-5 human fetal lung fibroblasts, and Detroit 551 human fetal skin fibroblasts. CF cells were obtained with informed consent from a skin biopsy taken from an adult cystic fibrosis patient. The Daley Lab provided dH1f fibroblasts, which were sub-cloned from fibroblasts produced by directed differentiation of the H1-OGN human ES cell line as previously described (Park et al., 2008). BGO1 hES cells were obtained from BresaGen (Athens, GA). H1 and H9 hES cells were obtained from WiCell (Madison, Wi).

RNA transfection

[00477] RNA transfections were carried out using RNAiMAX (Invitrogen) or TransIT-mRNA (Mirus Bio, Madison, WI) cationic lipid delivery vehicles. RNAiMAX was used for RiPS derivations, the RiPS-to-myogenic conversion, and for the multiple cell-type transfection experiment documented in Figures 3A-3E. All other transfections were performed with TransIT™-mRNA. For RNAiMAX transfections, RNA and reagent were first diluted in Opti-MEM™ basal media (Invitrogen). 100 ng/uL RNA was diluted 5x and 5 uL of RNAiMAX per microgram of RNA was diluted 10x, then these components were pooled and incubated 15 minutes at room temperature before being dispensed to culture media. For TransIT-mRNA transfections, 100 ng/uL RNA was diluted 10x in Opti-MEM™ and BOOST reagent was added (2 uL per microgram of RNA), then TransIT-mRNA was added (2 uL per microgram of RNA), and the RNA-lipid complexes were delivered to culture media after a 2-minute incubation at room temperature. RNA transfections were performed in Nutristem xeno-free hES media (Stemgent™, Cambridge, MA) for RiPS derivations, Dermal Cell Basal Medium plus Keratinocyte Growth Kit (ATCC) for keratinocyte experiments, and Opti-MEM™ plus 2% FBS for all other experiments described. The B18R interferon inhibitor (eBioscience, San Diego, CA) was used as a media supplement at 200 ng/mL.

qRT-PCR of interferon-regulated genes

[00478] Transfected and control 6-well cultures were washed with PBS and lysed *in situ* using 400 uL CellsDirect resuspension buffer/lysis enhancer (Invitrogen) per well, and 20 uL of each lysate was taken forward to a 50 uL reverse transcription reaction using the VILO cDNA synthesis kit (Invitrogen). Completed reactions were purified on QIAquick columns (Qiagen), and analyzed in 20 uL qPCRs, each templated with ~10% of the total cDNA prep. The reactions were performed using SYBR FAST qPCR supermix (KAPA Biosystems) with 250 nM primers and a thermal profile including 35 cycles of (95°C 3 s; 60°C 20 s). The qPCR primer sequences used are given **Table 5**.

Table 5: Primers for qRT-PCR analysis of interferon-regulated genes (SEQ ID NOs: 1467-1480, respectively, in order of appearance).

Transcript	Forward Primer	Reverse Primer
GAPDH	GAAGGCTGGGGCTCATTT	CAGGAGGCATTGCTGATGAT
IFNA	ACCCACAGCCTGGATAACAG	ACTGGTTGCCATCAAACTCC

IFNB	CATTACCTGAAGGCCAAGGA	CAGCATCTGCTGGTTGAAGA
IFIT1	AAAAGCCCACATTTGAGGTG	GAAATTCCTGAAACCGACCA
OAS1	CGATCCCAGGAGGTATCAGA	TCCAGTCCTCTTCTGCCTGT
PKR	TCGCTGGTATCACTCGTCTG	GATTCTGAAGACCGCCAGAG
RIG-I	GTTGTCCCCATGCTGTTCTT	GCAAGTCTTACATGGCAGCA

Reprogramming to pluripotency

[00479] Gamma-irradiated human neonatal fibroblast feeders (GlobalStem, Rockville, MD) were seeded at 33,000 cells/cm2. Nutristem™ media was used during the reprogramming phase of these experiments. Media was replaced daily, four hours after transfection, and supplemented with 100 ng/mL bFGF (Stemgent) and 200 ng/mL B18R before use. Where applied, VPA was added to media at 1 mM final concentration on days 8-15 of reprogramming. Low-oxygen culture experiments were carried out in a NAPCO 8000 WJ incubator (Thermo Scientific) supplied by NF300 compressed nitrogen cylinders (Airgas, Radnor, PA). Media were equilibrated at 5% O_2 for approximately 4 hours before use. Cultures were passaged using TrypLE Select recombinant protease (Invitrogen). Y27632 ROCK inhibitor (Watanabe et al., 2007) was purchased from Stemgent and included at 10 uM in recipient plates until the next media change, except where otherwise indicated. The daily RNA dose applied in the RiPS derivations was 1200 ng per well (6-well plate format) or 8 ug to a 10-cm dish.

[00480] For the RNA *vs.* retrovirus trial, both arms of the experiment were started with the same number of dH1f cells, and the passaging of the cultures was synchronized. Starting cultures were seeded with 100,000 cells in individual wells of a 6-well plate using fibroblast media (DMEM+10% FBS). The following day (day 1) KMOS RNA transfections were initiated in the RNA plate, and the viral plate was transduced with a KMOS retroviral cocktail (MOI=5 for each virus). All wells were passaged on day 6, using split ratios of 1:6 for the RNA wells and 1:3 for the virus wells. The conditions applied in the RNA arm of the trial were as in the initial RiPS derivation, including the use of Nutristem™ supplemented with 100 ng/mL bFGF, 5% O2 culture, and human fibroblast feeders. Ambient oxygen tension and other conventional iPS derivation conditions were used in the viral arm, the cells being grown in fibroblast media without feeders until the day 6 split, then being replated onto CF1 MEF feeders (GlobalStem) with a switch to hES media based on Knockout Serum Replacement (Invitrogen) supplemented with 10 ng/mL bFGF.

Culture of RiPS cell colonies

[00481] Emerging RiPS cell colonies were picked and clonally transferred to MEF-coated 24-well plates (Nunc, Rochester, NY) with standard hES medium containing 5 uM Y27632 (BioMol, Plymouth Meeting, PA). The hES media comprised DMEM/F12 supplemented with 20% Knockout Serum Replacement (Invitrogen), 10 ng/mL of bFGF (Gembio, West Sacramento, CA), 1x non-essential amino acids (Invitrogen), 0.1mM β-ME (Sigma), 1mM L-glutamine (Invitrogen), plus antibiotics. Clones were mechanically passaged once more to MEF-coated 6-well plates (Nunc), and

then expanded using enzymatic passaging with collagenase IV (Invitrogen). For RNA and DNA preparation, cells were plated onto hES-qualified Matrigel™ (BD Biosciences) in mTeSR (Stem Cell Technologies, Vancouver, BC), and further expanded by enzymatic passaging using dispase (Stem Cell Technologies).

Immunostaining of pluripotency markers

[00482] For fixed-cell imaging, RiPS colonies were mechanically picked and plated onto MEF feeders in black 96-well plates (Matrix Technologies, Maumee, OH). Two days post-plating, cells were washed with PBS and fixed in 4% paraformaldehyde for 20 minutes. After 3 PBS washes, cells were treated with 0.2% Triton X™ (Sigma) in PBS for 30 minutes to allow nuclear permeation. Cells were washed 3x in PBS and blocked in blocking buffer containing 3% BSA (Invitrogen) and 5% donkey serum (Sigma) for 2 hours at room temperature. After three PBS washes, cells were stained in blocking buffer with primary and conjugated antibodies at 4°C overnight. After washing 3x with PBS, cells were stained with secondary antibodies and 1 ug/mL Hoechst 33342 (Invitrogen) in blocking buffer for 3 hours at 4°C or for 1 hour at room temperature, protected from light. Cells were washed 3x with PBS before visualization. The following antibodies were used, at 1:100 dilution: TRA-1-60-Alexa Fluor™ 647, TRA-1-81-Alexa Fluor™ 488, SSEA-4-Alexa Fluor™ 647, and SSEA-3-Alexa 488 (BD Biosciences). Primary OCT4 and NANOG antibodies (Abcam, Cambridge, MA) were used at 0.5 ug/mL, and an anti-rabbit IgG Alexa Fluor™ 555 (Invitrogen) was used as the secondary. Images were acquired with a Pathway 435 bioimager (BD Biosciences) using a 10x objective. Live imaging was performed as described previously (Chan et al., 2009). Briefly, wells were stained by adding 1:100-diluted TRA-1-60-Alexa 647 and SSEA-4-Alexa 555 antibodies (BD Biosciences) to culture media. After 1.5 hours, Hoechst 33342 was added at a final concentration of 0.25 ug/mL, and wells were incubated for an additional 30 minutes. Wells were washed 3x with DMEM/F12 base media lacking phenol red, and imaged in hES media lacking phenol red. Images were acquired with a Pathway 435 bioimager using 4x and 10x objectives. Post-acquisition image processing and analysis was performed using Adobe Photoshop for pseudocoloring and ImageJ (http://rsbweb.nih.gov/ij) for flat-field correction, background subtraction, and colony quantitation.

[00483] For pluripotency factor time course experiments, transfected human epidermal keratinocytes were trypsinized, washed with PBS, and fixed in 4% paraformaldehyde for 10 minutes. Fixed cells were washed with 0.1M glycine, then blocked and permeabilized in PBS/0.5% saponin/1% goat serum (Rockland Immunochemicals, Gilbertsville, PA) for 20 minutes. Cells were incubated for 1 hour at room temperature with 1:100 diluted primary antibodies for KLF4, OCT4, SOX2 (Stemgent), washed, then for 45 minutes at room temperature with 1:200-diluted DyLight™ 488-labeled secondary antibodies (goat anti-mouse IgG+IgM and goat anti-rabbit IgG). Cells suspended in PBS were analyzed by flow cytometry.

Gene expression analysis

[00484] RNA was isolated using the RNeasy™ kit (Qiagen) according to the manufacturer's instructions. First-strand cDNA was primed with oligo(dT) primers and qPCR was performed with primer sets as described previously (Park et al., 2008), using Brilliant SYBR Green master mix (Stratagene, La Jolla, CA). For the microarray analysis, RNA probes were prepared and hybridized to Human Genome U133 Plus 2.0 oligonucleotide microarrays (Affymetrix, Santa Clara, CA) per the manufacturer's instructions. Arrays were processed by the Coriell Institute Genotyping and Microarray Center (Camden, NJ). Microarray data will be uploaded to the GEO database. Gene expression levels were normalized with the Robust Multichip Average (RMA) algorithm. Unsupervised hierarchical clustering was performed using the Euclidean distance with average linkage method. The similarity metric for comparison between different cell lines is indicated on the height of cluster dendrogram.

Bisulfite sequencing

[00485] DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's protocol. Bisulfite treatment of genomic DNA was carried out using EZ DNA Methylation™ Kit (Zymo Research, Orange, CA) according to the manufacturer's protocol. For pyrosequencing analysis, the bisulfite treated DNA was first amplified by HotStar Taq Polymerase (Qiagen) for 45 cycles of (95°C 30 s; 53°C 30 s; 72°C 30 s). The analysis was performed by EpigenDx using the PSQ™96HS system according to standard procedures using primers that were developed by EpigenDx for the CpG sites at positions (-50) to (+96) from the start codon of the OCT4 gene.

Tri-lineage differentiation

[00486] Embryoid body (EB) hematopoietic differentiation was performed as previously described (Chadwick et al., 2003). Briefly, RiPS cells and hES cell controls were passaged with collagenase IV and transferred (3:1) in differentiation medium to 6-well low-attachment plates and placed on a shaker in a 37°C incubator overnight. Starting the next day, media was supplemented with the following hematopoietic cytokines: 10 ng/mL of interleukin-3 (R&D Systems, Minneapolis, MN) and interleukin-6 (R&D), 50 ng/mL of G-CSF (Amgen, Thousand Oaks, CA) and BMP-4 (R&D), and 300 ng/mL of SCF (Amgen) and Flt-3 (R&D). Media was changed every 3 days. On day 14 of differentiation, EBs were dissociated with collagenase B (Roche, Indianapolis, IN). 2x104 differentiated cells were plated into methylcellulose H4434 (Stem Cell Technologies) and transferred using a blunt needle onto 35mm dishes (Stem Cell Technologies) in triplicate and incubated at 37°C and 5°CO2 for 14 days. Colony Forming Units (CFUs) were scored based on morphological characteristics.

[00487] For neuronal differentiation, cells were differentiated at 70% confluency as a monolayer in neuronal differentiation medium (DMEM/F12, Glutamax 1%, B27-Supplement 1%, N2-Supplement 2%, P/S 1% and noggin 20ng/ml). After 7 days neuronal structures were visible. For endoderm differentiation (AFP stain), cells were differentiated as a monolayer in endoderm differentiation medium (DMEM, B27(-RA) and 100 ng/ml activin-a) for 7 days, then switched to

growth medium (DMEM, 10% FBS, 1% P/S) and continued differentiation for 7 days. Primary antibodies used in immunostaining were as follows: Anti-β-Tubulin III (Tuj1) rabbit anti-human (Sigma, St. Louis, MO), 1:500; AFP (h-140) rabbit polyclonal IgG, (Santa Cruz Biotechnology, Santa Cruz, CA), 1:100 dilution. All secondary antibodies were conjugated to Alexa Fluor™ 488, Alexa Fluor™ 594 and raised in donkey.

[00488] For cardiomyocyte differentiation, colonies were digested at 70% confluency using dispase and placed in suspension culture for embryoid body (EB) formation in differentiation medium (DMEM, 15% FBS, 100 uM ascorbic acid). After 11 days, EBs were plated to adherent conditions using gelatin and the same medium. Beating cardiomyocytes were observed 3 days after replating.

[00489] For the teratoma assay, $2.5x10^6$ cells were harvested, spun down, and all excess media was removed. In a 20-week old female SCID mouse, the capsule of the right kidney was gently elevated, and one droplet of concentrated cells was inserted under the capsule. At week 6, when adequate tumor size was observed, the tumor was harvested, fixed in 4% PFA, run through an ethanol gradient, and stored in 70% ethanol. Specimens were sectioned and H&E staining. Slides were imaged with a Leica light microscope.

Myogenic differentiation of RiPS cells

[00490] Validated RiPS cells were plated into wells coated with 0.1% gelatin (Millipore, Billerica, MA), and cultured in DMEM+10% FBS for 4 weeks with passaging every 4-6 days using trypsin. The culture media was switched to Opti-MEM+2% FBS, and the cells were transfected with modified RNA encoding either murine MYOD or GFP the following day, and for the following two days. Media was supplemented with B18R, and replaced 4 hours after each transfection. After the third and final transfection, the media was switched to DMEM+3% horse serum, and cultures were incubated for a further 3 days. Cells were then fixed in 4% PFA and immuno-stained as previously described (Shea et al., 2010). The percentage of myogenin-positive nuclei/total nuclei and nuclei/MyHC-positive myotubes was quantified, with a minimum of 500 nuclei counted per condition.

[00491] Thus far, the reprogramming of differentiated cells to pluripotency shows great utility as a tool for studying normal cellular development, while also having the potential for generating patient-specific induced pluripotent stem (iPS) cells that can be used to model disease, or to generate clinically useful cell types for autologous therapies aimed at repairing deficits arising from injury, illness, and aging. Induction of pluripotency was originally achieved by Yamanaka and colleagues by enforced expression of four transcription factors, KLF4, c-MYC, OCT4, and SOX2 (KMOS) using retroviral vectors (Takahashi et al., 2007; Takahashi and Yamanaka, 2006).

[00492] A formidable obstacle to therapeutic use of iPS cells has been presented by the requirement for viral integration into the genome. The search for ways to induce pluripotency without incurring genetic change has become the focus of intense research effort. Towards this end, attempts to derive iPS cells using excisable lentiviral and transposon vectors, or through repeated application of

transient plasmid, episomal, and adenovirus vectors have been made (Chang et al., 2009; Kaji et al., 2009; Okita et al., 2008; Stadtfeld et al., 2008; Woltjen et al., 2009; Yu et al., 2009). Human iPS cells have also been derived using two DNA-free methods: serial protein transduction with recombinant proteins incorporating cell-penetrating peptide moieties (Kim et al., 2009; Zhou et al., 2009), and transgene delivery using the Sendai virus, which has a completely RNA-based reproductive cycle (Fusaki et al., 2009).

[00493] Considerable limitations accompany the non-integrative iPS derivation strategies devised thus far. For example, DNA transfection-based methodologies still entail risk of genomic recombination or insertional mutagenesis, even though they are supposedly safer than viral-based delivery methods. In the protein-based strategies thus far derived, the recombinant proteins used are difficult and challenging to generate and purify in the quantities required, and result in even lower efficiencies of pluripotent stem cell generation that conventional viral-based methods (Zhou et al., 2009). Use of Sendai virus requires stringent steps to purge reprogrammed cells of replicating virus, and the sensitivity of the viral RNA replicase to transgene sequence content can further limit the generality of this reprogramming vehicle (Fusaki et al., 2009). Importantly, the methods discussed that rely on repeat administration of transient vectors, whether DNA or protein-based, have shown very low reprogramming and iPS derivation efficiencies (Jia et al., 2010; Kim et al., 2009; Okita et al., 2008; Stadtfeld et al., 2008; Yu et al., 2009; Zhou et al., 2009), presumably due, without wishing to be bound or limited by theory, to weak or inconstant expression of reprogramming factors.

[00494] As demonstrated herein, the inventors have discovered and shown that repeated administration of synthetic, modified messenger RNAs that incorporate novel modifications designed to bypass innate cellular anti-viral responses can reprogram differentiated human cells to pluripotency with conversion efficiencies and kinetics vastly and unexpectedly superior to established protein- and viral-based protocols. Accordingly, described herein are methods and compositions demonstrating that this non-mutagenic, efficient, and highly controllable technology is applicable to a wide range of cellular engineering tasks involving altering cellular developmental potentials, such as the reprogramming of differentiated cells, and the differentiation of reprogrammed cells to a differentiated cell type, such as RNA-iPS (RiPS)-derived fibroblasts to terminally differentiated myogenic cells.

Development of synthetic, modified RNAs for directing cell fate

[00495] mRNA was manufactured using *in vitro* transcription (IVT) reactions templated by PCR amplicons (**Figure 1**). To promote efficient translation and boost RNA half-life in the cytoplasm, a 5' guanine cap was incorporated by inclusion of a synthetic cap analog in the IVT reactions (Yisraeli et al., 1989). Within the IVT templates described herein, the open reading frame (ORF) of the gene of interest is flanked by a 5' untranslated region (UTR) containing a strong Kozak translational initiation signal, and an alpha-globin 3' UTR terminating with an oligo(dT) sequence for templated addition of a polyA tail.

[00496] Cytosolic delivery of mRNA into mammalian cells can be achieved using electroporation or by complexing the RNA with a cationic vehicle to facilitate uptake by endocytosis (Audouy and Hoekstra, 2001; Elango et al., 2005; Holtkamp et al., 2006; Van den Bosch et al., 2006; Van Tendeloo et al., 2001). The latter approach was utilized by the inventors as it would allow for repeated transfection to sustain ectopic protein expression over the days to weeks required for cellular reprogramming. In experiments in which synthetic RNA encoding GFP was transfected into murine embryonic fibroblasts and human epidermal keratinocytes, high, dose-dependent cytotoxicity was noted, which was not attributable to the cationic vehicle, and which was exacerbated on repeated transfections. These experiments demonstrated a serious impediment to achieving sustained protein expression by repeated mRNA transfection.

[00497] It is has been reported that exogenous single-stranded RNA (ssRNA) activates antiviral defenses in mammalian cells through interferon and NF-κB dependent pathways (Diebold et al., 2004; Hornung et al., 2006; Kawai and Akira, 2007; Pichlmair et al., 2006; Uematsu and Akira, 2007). In order to increase the sustainability of RNA-mediated protein expression, approaches were sought to reduce the immunogenic profile of the synthetic RNA. The co-transcriptional capping technique yields a significant fraction of uncapped IVT product bearing 5' triphosphates, which has been reported to trigger the ssRNA sensor RIG-I (Hornung et al., 2006; Pichlmair et al., 2006), and have also been reported to activate PKR, a global repressor of cellular protein translation (Nallagatla and Bevilacqua, 2008). However, treatment of the synthesized RNA with a phosphatase only resulted in modest reductions in the observed cytotoxicity upon repeated transfections.

[00498] Eukaryotic mRNA is extensively modified *in vivo*, and the presence of modified nucleobases has been shown to reduce signaling by RIG-I and PKR, as well as by the less widely expressed but inducible endosomal ssRNA sensors TLR7 and TLR8 (Kariko et al., 2005; Kariko et al., 2008; Kariko and Weissman, 2007; Nallagatla and Bevilacqua, 2008; Nallagatla et al., 2008; Uzri and Gehrke, 2009). In an attempt to further reduce innate immune responses to transfected RNA, mRNAs were synthesized incorporating modified ribonucleoside bases. Complete substitution of either 5-methylcytidine (5mC) for cytidine or pseudouridine (psi) for uridine in GFP-encoding transcripts markedly improved viability and increased ectopic protein expression.

[00499] However, the most significant improvements in viability and protein expression were observed when both 5-methylcytidine and pseudouridine were used together (**Figure 2A** and **2H**). It was discovered that these modifications dramatically attenuated interferon signaling as revealed by qRT-PCR for a panel of interferon response genes, although residual upregulation of some interferon targets was still detected (**Figures 2B-2G**). Innate cellular anti-viral defenses can self-prime through a positive-feedback loop involving autocrine and paracrine signaling by Type I interferons (Randall and Goodbourn, 2008). It was found that media supplementation with a recombinant version of B18R protein, a Vaccinia virus decoy receptor for Type I interferons (Symons et al., 1995), further increased cellular viability following RNA transfection, especially in some cell types. It was discovered that

synthesis of RNA with a combination of both modified 5-methylcytidine and pseudouridine ribonucleotides and phosphatase treatment (herein termed "synthetic, modified RNAs"), combined with media supplementation with the interferon inhibitor B18R allowed high, dose-dependent levels of protein expression (**Figure 2H**).

[00500] It was discovered that transfection of synthetic, modified RNA encoding GFP into six different human cell types resulted in highly penetrant expression (50-90% positive cells), and demonstrated the applicability of these novel methods and compositions to diverse cell types (**Figure 3A**). Simultaneous delivery of synthetic, modified RNAs encoding cytosolic-localized, and nuclear-localized fluorescent proteins into keratinocytes revealed that generalized co-expression of multiple proteins could be achieved in mammalian cells, and that the resulting proteins were correctly localized to the cytosol and nucleus, respectively.

[00501] Ectopic protein expression after RNA transfection is transient owing to RNA and protein degradation and the diluting effect of cell division. To establish the kinetics and persistence of protein expression, synthetic, modified RNA encoding GFP variants designed for high and low protein stability (Li et al., 1998) were synthesized and transfected into keratinocytes. Time-course analysis by flow cytometry showed that protein expression persisted for several days for the high-stability variant, but peaked within 12 hours and decayed rapidly thereafter for the destabilized GFP (**Figures 3B** and **3D**). These results indicated that a repetitive transfection regimen would be necessary in order to sustain high levels of ectopic expression for short-lived proteins over an extended time course.

[00502] To assess this and further address the impact of repeated RNA transfection on cell growth and viability, BJ fibroblasts were transfected daily for 10 days with either unmodified, or synthetic, modified RNAs encoding GFP. It was discovered that daily transfection with synthetic, modified RNA permitted sustained protein expression without substantially compromising the viability of the culture beyond a modest reduction in growth kinetics that was attributable to the transfection reagent vehicle (**Figures 2J** and **3C**). Microarray analysis established that prolonged daily transfection with synthetic, modified RNA did not significantly alter the molecular profile of the transfected cells, although a modest upregulation of a number of interferon response genes was noted, consistent with the fact that the modifications described herein did not completely abrogate interferon signaling (**Figures 2B-2G, Figure 3E**). In complete contrast, repeated transfections with unmodified RNA severely compromised the growth and viability of the culture through, in part, elicitation of a massive interferon response (**Figures 2B-2G, Figure 3E**), demonstrating that the use of unmodified RNA is not a viable strategy for sustaining long-term polypeptide expression in cells (**Figure 2J**).

[00503] To determine if modified RNAs could be used to directly alter cell fate, synthetic, modified RNA was synthesized encoding the myogenic transcription factor MYOD (Davis et al., 1987) and transfected into murine C3H10T1/2 cells over the course of 3 days, followed by continued culturing in a low serum media for an additional 3 days. The emergence of large, multi-nucleated

myotubes that stained positive for the myogenic markers myogenin and myosin heavy chain (MyHC) provided proof that transfection with synthetic, modified RNAs could be utilized to efficiently direct cell fate (**Figure 2K**).

Generation of induced pluripotent stem cells using modified RNAs

[00504] The determination of whether induced pluripotent stem cells (iPS) could be derived using synthetic, modified RNAs was next attempted. To this end, synthetic, modified RNAs encoding the four canonical Yamanaka factors, KLF4 (K), c-MYC (M), OCT4 (O), and SOX2 (S), were synthesized, transfected into cells. It was discovered that the synthetic, modified RNAs encoding transcription factors yielded robust protein expression that localized to the nucleus. Time-course analysis monitored by flow cytometry yielded expression kinetics and stability similar to destabilized GFP (**Figure 3B** and **3D**), demonstrating rapid turnover of these transcription factors (**Figures 4A-4C**). From this, it was concluded that daily transfections would be required to maintain sufficient expression of the Yamanaka factors during long-term, multi-factor reprogramming regimens.

[00505] A protocol to ensure sustained high-level protein expression with daily transfection was next discovered by exploring a matrix of conditions encompassing a variety of different transfection reagents, culture media, feeder cell types, and RNA doses. Long-term reprogramming experiments were initiated with human ES-derived dH1f fibroblasts, which display relatively efficient viral-mediated iPS cell conversion (Chan et al., 2009; Park et al., 2008). Low-oxygen (5% O2) culture conditions and a KMOS stoichiometry of 1:1:3:1 were also employed, as these have been reported to promote efficient iPS conversion in viral-based methods (Kawamura et al., 2009; Papapetrou et al., 2009; Utikal et al., 2009; Yoshida et al., 2009). Synthetic, modified RNA encoding a short half-life nuclear GFP was spiked into the KMOS RNA cocktail to allow visualization of continued protein expression from modified RNA during the course of the experiment. Experiments conducted in this manner revealed widespread transformation of fibroblast morphology to a compact, epithelioid morphology within the first week of synthetic, modified RNA transfection, which was followed by emergence of canonical hES-like colonies with tight morphology, well-defined borders, and prominent nucleoli. RNA transfection was terminated on day 17, and three days later colonies were mechanically picked and expanded to establish 14 prospective iPS lines, designated dH1f-RiPS (RNA-derived iPS) 1-14.

[00506] It was next attempted to reprogram somatically-derived cells to pluripotency using a similar reprogramming regimen. A five-factor cocktail including a modified RNA encoding LIN28 (KMOSL) (Yu et al., 2007) was employed and the media was supplemented with valproic acid (VPA), a histone deacetylase inhibitor, which has been reported to increase reprogramming efficiency (Huangfu et al., 2008). Four human cell types were tested: Detroit 551 (D551) and MRC-5 fetal fibroblasts, BJ post-natal fibroblasts, and fibroblast-like cells cultured from a primary skin biopsy taken from an adult cystic fibrosis patient (CF cells). Daily transfection with the modified RNA KMOSL cocktail gave rise to numerous hES-like colonies in the D551, BJ, and CF cultures that were

mechanically picked at day 18, while MRC-5-derived colonies were picked at day 25. Multiple RiPS colonies were expanded for each of the somatic lines, and immunostaining confirmed the expression of hES markers TRA-1-60, TRA-1-81, SSEA3, SSEA4, OCT4, and NANOG in all the RiPS lines examined. Three RiPS cell clones from each of these four derivations were analyzed and confirmed to originate from the seeded somatic cells by DNA fingerprinting, and all presented normal karyotypes. In the experiments described above, the transfected fibroblast cultures were passaged once at an early time point (day 6 or 7) in order to promote fibroblast proliferation, which has been shown to facilitate reprogramming (Hanna et al., 2009). However, in independent experiments, RiPS cells were also derived from BJ and Detroit 551 fibroblasts in the absence of cell passaging, indicating that this was not required for modified RNA iPS-derivation (**Figure 6**).

Molecular characterization and functional potential of RiPS cells

[00507] A number of molecular and functional assays were performed to assess whether the RiPS cells described herein had been reprogrammed to pluripotency (**Table 6**). Multiple RiPS lines derived from each of the five starting cell types were evaluated by quantitative RT-PCR (qRT-PCR), and all demonstrated robust expression of the pluripotency-associated transcripts OCT4, SOX2, NANOG, and hTERT (**Figure 7A**). RiPS clones derived from dH1f, MRC5, BJ, and CF fibroblasts were further analyzed by bisulfite sequencing, which revealed extensive demethylation of the OCT4 locus relative to the parental fibroblasts, an epigenetic state equivalent to human ES cells (**Figure 7B**).

Table 6: Pluripotency validation assays performed in this study.

Immunostaining#	qRT-PCR	Bisulfite Sequencing$^{\Omega}$	Microarray	Developmental Potential	
				In vitro	Teratoma
dH1F-RiPS-1.3	dH1F-RiPS-1.2	dH1F-RiPS-1.2	dH1F-RiPS-1.2	dH1F-RiPS-1.2^†ø*	dH1F-RiPS-1.3
dH1F-RiPS-1.6	dH1F-RiPS-1.3	dH1F-RiPS-1.3	dH1F-RiPS-1.3	dH1F-RiPS-1.6^ø	dH1F-RiPS-1.5
dH1F-RiPS-1.13	dH1F-RiPS-1.6	dH1F-RiPS-1.6	dH1F-RiPS-1.6	dH1F-RiPS-1.13^ø	dH1F-RiPS-1.6
BJ-RiPS-1.1	dH1F-RiPS-1.7	BJ-RiPS-1.2	dH1F-RiPS-1.7	dH1F-RiPS-1.14^ø	dH1F-RiPS-1.7
BJ-RiPS-1.2	BJ-RiPS-1.1	BJ-RiPS-1.3	BJ-RiPS-1.1	MCR5-RiPS-1.8^†*	dH1F-RiPS-1.11
BJ-RiPS-1.3	BJ-RiPS-1.2	MCR5-RiPS-1.8	BJ-RiPS-1.2	MCR5-RiPS-1.9^†*	BJ-RiPS-1.1
MCR5-RiPS-1.2	BJ-RiPS-1.3	MCR5-RiPS-1.9	BJ-RiPS-1.3	MCR5-RiPS-1.11^*	BJ-RiPS-1.2
MCR5-RiPS-1.3	MCR5-RiPS-1.8	MCR5-RiPS-1.11	MCR5-RiPS-1.8	BJ-RiPS-1.1^†ø*	CF-RiPS-1.2
MCR5-RiPS-1.11	MCR5-RiPS-1.9	CF-RiPS-1.2	MCR5-RiPS-1.9	BJ-RiPS-1.2^†ø*	
CF-RiPS-1.2	MCR5-RiPS-1.11	CF-RiPS-1.3	MCR5-RiPS-1.11	BJ-RiPS-1.3^†*	
CF-RiPS-1.3	CF-RiPS-1.2	CF-RiPS-1.4	CF-RiPS-1.2	CF-RiPS-1.2^†*	
CF-RiPS-1.4	CF-RiPS-1.3		CF-RiPS-1.3	CF-RiPS-1.3^†*	
D551-RiPS-1.1	CF-RiPS-1.4		CF-RiPS-1.4	CF-RiPS-1.4^ø*	
D551-RiPS-1.2	D551-RiPS-1.1			D551-RiPS-1.1^†*	
D551-RiPS-1.3	D551-RiPS-1.2			D551-RiPS-1.2^*	
	D551-RiPS-1.3			D551-RiPS-1.3^*	

Table 6 shows the RiPS clones that were validate in each assay. # Validated for immuno-staining for all of TRA-1-60, TRA-1-80, SSEA3, SSEA4, OCT4, NANOG. Ω Demethylation of the OCT4 promoter. *In vitro* differentiation including ^embryoid body formation, øtrilineage by directed differentiation, † beating cardiomyocytes, and * blood formation by CFC assays in methylcellulose.

[00508] To gain more global insight into the molecular properties of RiPS cells, gene expression profiles of RiPS clones from multiple independent derivations were generated and compared to fibroblasts, human embryonic stem (ES) cells, and virally-derived iPS cell lines. These analyses revealed that all synthetic, modified RNA-derived iPS clones examined had a molecular signature that very closely recapitulated that of human ES cells while being highly divergent from the profile of the parental fibroblasts (**Figures 7C-7H**). Importantly, pluripotency-associated transcripts including SOX2, REX1, NANOG, OCT4, LIN28 and DNMT3B were substantially upregulated in the RiPS cells compared to the parental fibroblast lines to levels comparable to human ES cells (**Figures 7C-7H**). Furthermore, when the transcriptional profiles were subjected to unsupervised hierarchical clustering analysis, all RiPS clones analyzed clustered more closely to human ES cells than did virally-derived iPS cells, indicating that synthetic, modified RNA-derived iPS cells more fully recapitulated the molecular signature of human ES cells (**Figure 7I**).

[00509] To evaluate the developmental potential of RiPS cells, embryoid bodies (EBs) were generated from multiple clones representing five independent RiPS derivations. Beating cardiomyocytes were observed for vast majority of the EBs (**Table 6**). Mesodermal potential was further evaluated in methylcellulose assays which showed that all lines tested were able to differentiate into hematopoietic precursors capable of giving rise to colony numbers and a spectrum of blood colony types comparable to human ES cells (**Figure 8**, **Table 6**). A subset of clones was further plated onto matrigel and differentiated into Tuj1-positive neurons (ectoderm), and alpha-fetoprotein-positive endodermal cells (**Table 6**). Finally, tri-lineage differentiation potential was confirmed *in vivo* by the formation of teratomas from dH1F-, CF- and BJ-RiPS cells, that histologically revealed cell types of the three germ layers (**Table 6**).

[00510] Taken together, these data demonstrate by the most stringent molecular and functional criteria available in regard to human pluripotent cells (Chan et al., 2009; Smith et al., 2009), that the synthetic, modified RNA-derived iPS clones from multiple independent derivations described herein were reprogrammed to pluripotency, and closely recapitulated the functional and molecular properties of human ES cells. Significantly, these synthetic, modified RNA-derived iPS clones had molecular properties more similar to human ES cells than did cells that were reprogrammed using standard, viral-based methods.

Modified RNAs generate iPS cells at very high efficiency

[00511] During the course of the experiments, surprisingly high reprogramming efficiencies and rapid kinetics of iPS cell generation using the synthetic, modified RNAs described herein were observed. To quantify the efficiency of RiPS derivation more thoroughly, a number of reprogramming experiments were undertaken and results quantitated based on the expression of the iPS-specific markers TRA-1-60 and TRA-1-81, (Chan et al., 2009; Lowry et al., 2008). In one set of experiments, BJ fibroblasts transfected with a five-factor modified RNA cocktail (KMOSL), this time without the use of VPA, demonstrated an iPS conversion efficiency of over 2%, which is two orders of magnitude

higher than typically reported for virus-based derivations (**Figure 9A**, **Table 7**). Moreover, in contrast to virus-mediated BJ-iPS derivations, in which iPS colonies typically take around 4 weeks to emerge, by day 17 of RNA transfection the plates had already become overgrown with ES-like colonies.

Table 7: Quantification of reprogramming efficiency.

Experiment	Cells plated	Split	Condition	Well fraction	Colonies/well	Efficiency (%)
BJ (KMOSL)	300,000	d7	Y27632-	1/24	249 ± 21	2.0
			Y27632+	1/24	326 ± 49	2.6
4-Factor (KMOS) *vs.* 5-Factor (KMOSL)	50,000	d6	4F 20% O_2	1/6	48 ± 18	0.6
			4F 5% O_2	1/6	228 ± 30	2.7
			5F 20% O_2	1/6	243 ± 42	2.9
			5F 5% O_2	1/6	367 ± 38	4.4
RNA *vs.* Virus (KMOS)	100,000	d6	Virus	1/3	13 ± 3.5	0.04
			RNA	1/6	229 ± 39	1.4

For each experimental condition, efficiency was calculated by dividing the average count of TRA-1-60-positive colonies per well by the initial number of cells plated, scaled to the fraction of cells replated in each well. Cultures were passaged at day 6 or 7 as indicated. The BJ experiment was started in a 10-cm dish, dH1f trials in individual wells of a 6-well plate. Colony counts are shown ± s.d., n=6, except in the RNA vs. Virus trial, where n=9 for virus, n=18 for RNA.

[00512] In another set of experiments, the contributions of low-oxygen culture and LIN28 to the efficiency of RiPS derivation were evaluated. The yield of TRA-1-60/TRA-1-81-positive colonies in the ambient (20%) oxygen condition was four-fold lower than in the cultures maintained at 5% O2 when using KMOS RNA, but this deficit was negated when LIN28 was added to the cocktail (**Table 7**). The highest conversion efficiency (4.4%), which is higher than any reported conversion efficiency, was observed when low-oxygen culture and the five-factor KMOSL cocktail were combined.

[00513] To directly compare the kinetics and efficiency of the RiPS derivation protocol against an established viral protocol, an experiment in which dH1f fibroblasts were transfected with KMOS synthetic, modified RNAs, or transduced with KMOS retroviruses in parallel was conducted. As had been observed in the previous experiments described herein, ES-like colonies began to emerge by day 13 from the synthetic, modified RNA-transfected cultures, and the plates became overgrown with ES-like colonies by the 16th and final day of transfection. These synthetic, modified RNA-derived cultures were therefore fixed for analysis on day 18 (**Figure 9C**). Notably, at this time, no ES-like colonies had appeared in the retrovirally transduced cultures, and colonies only began to emerge on the 24th day post-transduction, which is a time point consistent with previous reports describing iPS derivations by retroviruses (Lowry et al., 2008; Takahashi et al., 2007). These retroviral-derived cultures were fixed for analysis on day 32. Both arms of the experiment were then immunostained and TRA-1-60-positive colonies were counted. These experiments revealed that the kinetics of modified

RNA iPS derivation were almost twice as fast as retroviral iPS derivation. Further, and importantly, iPS derivation efficiencies were 1.4% for synthetic, modified RNA cultures, and only 0.04% for retroviral cultures, corresponding to a surprising 36-fold higher conversion efficiency with the synthetic, modified RNA compositions and protocols (**Figure 9D**, **Table 7**). Thus, by the combined criteria of colony numbers and kinetics of reprogramming, the efficiency of synthetic, modified RNA iPS derivation unexpectedly greatly exceeds that of conventional retroviral approaches.

Utilization of synthetic, modified RNA to direct differentiation of pluripotent RiPS cells to a terminally-differentiated cell fate.

[00514] To realize the promise of iPS cell technology for regenerative medicine or disease modeling, it is imperative that the multi-lineage differentiation potential of pluripotent cells be harnessed. Although limited progress has been made in directing the differentiation of pluripotent ES cells to various lineages by modulating the extracellular cytokine milieu, such protocols remain inefficient. Given the high efficiency of iPS derivation by the novel synthetic, modified RNAs and methods thereof described herein, whether this technology could also be utilized to redirect pluripotent or multipotent cells towards differentiated cell fates was also determined. To test this, one of the validated RiPS lines described herein was subjected to an *in vitro* differentiation protocol in which FGF was withdrawn, serum added, and the cells plated onto gelatin (**Figures 10A-10B**). Cells obtained under these conditions were subjected to three consecutive days of transfection with a MYOD-encoding synthetic, modified RNA to provoke myogenic differentiation. The cells were then cultured an additional three days and then immunostained for the myogenic markers myogenin and MyHC, which revealed a high percentage of large multi-nucleated myogenin and MyHC double positive myotubes (**Figures 10A-10B**).

[00515] Taken together, the experiments described herein provide clear proof that synthetic, modified RNAs can be used to both reprogram cells to a pluripotent state at high and unexpected efficiencies, and also direct the fate of such cells and other pluripotent or multipotent cells to cells having lower developmental potential, such as a terminally differentiated somatic cell type.

Discussion

[00516] Described herein are novel compositions and technologies that use a combination of synthetic RNA modifications, and in some embodiments, a soluble interferon inhibitor, to overcome innate anti-viral responses and permit repeated transfections with RNA, thus enabling highly efficient alterations in cellular phenotypes and developmental potentials, such as highly efficient reprogramming of somatic cells to pluripotency, and directing the differentiation of pluripotent cells towards a desired lineage. The novel methodologies and compositions described herein offer several key advantages over established reprogramming techniques. By obviating the need to perform experiments under stringent biological containment, synthetic, modified RNA technology makes reprogramming accessible to a wider community of researchers. More fundamentally, the approaches

described herein allow protein stoichiometry to be regulated globally within cultures, while avoiding the stochastic variation of expression typical of integrating vectors, as well as the uncontrollable and undesired effects of viral silencing. Given the stepwise character of the phenotypic changes observed during pluripotency induction (Chan et al., 2009; Smith et al., 2010), individual transcription factors can play distinct, stage-specific roles during reprogramming. The unprecedented potential for temporal control over factor expression afforded by the technologies described herein can help researchers unravel these nuances, yielding further insights that can be applied to further enhance the efficiency and kinetics of reprogramming.

[00517] While the risk of mutagenesis is a major safety concern holding back clinical exploitation of induced pluripotency, other factors also play a role. It has become increasingly apparent that all iPS cells are not created equal with respect to epigenetic landscape and developmental plasticity (Hu et al., 2010; Miura et al., 2009). In this regard, the most stringent molecular and functional criteria for reprogramming human cells have been applied herein (Chan et al., 2009; Smith et al., 2009), to demonstrate that the iPS clones derived from synthetic, modified RNAs from multiple independent derivations were reprogrammed to pluripotency, and also closely recapitulated the functional and molecular properties of human ES cells. Significantly, as described herein, synthetic, modified RNA derived iPS cells more faithfully recapitulated the global transcriptional signature of human ES cells than retrovirally-derived iPS cells, indicating that the compositions and methods for RNA reprogramming described herein produce higher quality iPS cells, possibly owing, without wishing to be bound or limited by theory, to the fact that they are transgene-free.

[00518] The transient and non-mutagenic character of RNA-based protein expression can also deliver important clinical benefits, in some embodiments, outside the domain of lineage reprogramming and alteration of cellular developmental potential. The use of RNA transfection to express cancer or pathogen antigens for immunotherapy is already an active research area (Rabinovich et al., 2008; Rabinovich et al., 2006; Van den Bosch et al., 2006; Weissman et al., 2000), and the synthetic, modified RNA can be used, in some embodiments, to transiently express surface proteins, such as homing receptors, to target cellular therapies toward specific organs, tissues, or diseased cells (Ryser et al., 2008).

[00519] For tissue engineering to progress further, there is a pressing need for safe and efficient means to alter cellular fates. In terms of personalized medicine applications, iPS cells are a starting point for patient-specific therapies, and specification of clinically useful cell types is required to produce autologous tissues for transplantation or for disease modeling. Importantly, the inventors have demonstrated that the synthetic, modified RNA-based technologies described herein that enable highly efficient reprogramming, can are equally applicable to efficiently alter pluripotent cell fate to terminally differentiated fates without compromising genomic integrity. In light of these

considerations, the novel compositions and approaches described herein can become central enabling technology for cell-based therapies and regenerative medicine.

CLAIMS

1. An *in vitro or ex vivo* method of altering the developmental potential of a cell, the method comprising contacting the cell or progeny cells thereof with synthetic, modified RNAs encoding developmental potential altering factors OCT4, SOX2, KLF4, and c-MYC at least three times, wherein each cytosine of each of the synthetic, modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic, modified RNAs is replaced with pseudouridine.

2. The method of claim 1-4, wherein each of the synthetic, modified RNAs further comprises a 5' cap.

3. The method of claim 2, wherein the 5' cap is a 5' cap analog.

4. The method of claim 3, wherein the 5' cap analog is a 5' diguanosine cap.

5. The method of any one of claims 1-4, wherein each of the synthetic, modified RNAs does not comprise a 5' triphosphate.

6. The method of any one of claims 1-5, wherein each of the synthetic, modified RNAs further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof.

7. The method of any one of claims 1-6, further comprising a step of determining that the cell or progeny cells thereof maintain viability by measuring viability of the cell or progeny cells thereof, wherein viability of at least 50% of the contacted cell or progeny cells thereof indicates that the cell or progeny cells thereof maintain viability.

8. The method of any one of claims 1-7, further comprising a step of determining that the cell or progeny cells thereof does not have a significant increase in expression of Type I or Type II IFN by measuring expression of Type I or a Type II IFN in the contacted cell or progeny cells thereof, wherein less than three-fold increase in expression of Type I or Type II IFN in the contacted cell or progeny cells thereof compared to cells that have not been contacted with the synthetic, modified RNAs indicates that the cell or progeny cells thereof do not have a significant increase in expression of Type I or Type II IFN.

9. The method of claim 8, wherein measuring the expression of Type I or Type II IFN is performed by measuring expression of at least one IFN-signature gene selected from IFNα, IFNB1, IFIT, OAS1, PKR,

RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20, wherein a less than six-fold increase in expression of the at least one IFN-signature gene compared to said cell or progeny cells thereof prior to contacting the said cell or progeny cells thereof with the synthetic, modified RNAs.

10. The method of any one of claims 1-9, wherein the contacting of the cell or progeny cells thereof is performed *in vitro*.

11. An *in vitro or ex vivo* method of reprogramming a somatic cell into an induced pluripotent stem cell, the method comprising contacting the somatic cell or progeny cells thereof with synthetic, modified RNAs encoding reprogramming factors OCT4, SOX2, KLF4, and c-MYC at least five consecutive times, wherein each cytosine of each of the synthetic, modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic, modified RNAs is replaced with pseudouridine.

12. The method of claim 11, wherein the at least five consecutive times occur within 25 days.

13. The method of claims 11 or 12, wherein each of the synthetic, modified RNAs further comprises a 5' cap.

14. The method of claim 13, wherein the 5' cap is a 5' cap analog.

15. The method of claim 14, wherein the 5' cap analog is a 5' diguanosine cap.

16. The method of any one of claims 11-15, wherein each of the synthetic, modified RNAs does not comprise a 5' triphosphate.

17. The method of any one of claims 11-16, wherein each of the synthetic, modified RNAs further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof.

18. The method of any one of claims 11-17, wherein the somatic cell is a human somatic cell.

19. The method of any one of claims 11-18, further comprising a synthetic, modified RNA molecule encoding LIN28, wherein each cytosine of the synthetic, modified RNA encoding LIN28 is replaced with 5-methylcytosine and each uracil of the synthetic, modified RNA encoding LIN28 is replaced with pseudouridine.

20. The method of any one of claims 11-19, further comprising a step of determining that the somatic cell or progeny cells thereof maintain viability by measuring viability of the somatic cell or progeny cells thereof, wherein viability of at least 50% of the contacted somatic cell or progeny cells thereof indicates that the cells maintain viability.

21. The method of any one of claims 11-20, further comprising the step of determining that the reprogrammed somatic cell produced by the method has an increased likeness to the potency of an embryonic stem cell by subjecting the induced pluripotent stem cell or induced pluripotent stem cell population generated by the method to an unsupervised hierarchical cluster analysis and comparing it to a reference from an unsupervised cluster analysis of an induced pluripotent stem cell produced by viral expression of one or more of the reprogramming factors, exogenous protein introduction of one or more reprogramming factors, small molecule mediated expression or induction of one or more reprogramming factors, wherein if the reprogrammed somatic cell clusters more closely to an embryonic stem cell than it does to a the reference, it has an increased likeness to the potency of embryonic stem cell.

22. The method of any one of claims 11-21, further comprising a step of determining that the reprogrammed somatic cell or progeny cell thereof does not have a significant increase in expression of IFN by measuring expression of at least one IFN-signature gene in the reprogrammed somatic cell or progeny cell thereof, wherein if the increase in expression of the at least one IFN-signature gene is less than six-fold compared to a reference from a somatic cell prior to it being subjected to reprogramming indicates that the reprogrammed somatic cell or progeny cell thereof does not have a significant increase in expression of IFN.

23. The method of claim 22, wherein the IFN-signature gene is selected from the group consisting of IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20.

24. The method of any one of claims 11-23, wherein said somatic cell or progeny cells thereof are contacted under a low-oxygen condition.

25. The method of any one of claims 11-24, further comprising determining that the reprogrammed somatic cell or progeny thereof expresses sufficient levels of genes to determine pluripotency by measuring expression of at least two genes selected from the group consisting of SOX2, REX1, DNMT3B, TRA-1-60, TRA-1-81, SSEA3, SSEA4, OCT4, and NANOG and comparing the result to a reference from an embryonic stem cell, wherein if at least two of the genes is expressed at the level they are expressed in the embryonic stem

cell, it indicates that the reprogrammed somatic cell or progeny thereof expresses sufficient levels of genes to determine pluripotency.

26. The method of any one of claims 11-25, wherein said contacting of the somatic cell or progeny cells thereof is performed *in vitro*.

27. A cell comprising exogenously introduced synthetic, modified RNAs encoding a developmental potential altering factors OCT4, SOX2, KLF4, and c-MYC, wherein each cytosine of each of the synthetic, modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic, modified RNAs is replaced with pseudouridine.

28. The cell of claim 27, wherein the cell is a human cell.

29. The cell of claim 27, wherein the cell is not a human cell.

30. The cell of any one of claims 27-29, wherein said cell or its immediate precursor cell(s) has been subjected to at least three consecutive rounds of contacting with the exogenously introduced synthetic, modified RNAs encoding the developmental potential altering factors OCT4, SOX2, KLF4 and c-MYC.

31. The cell of any one of claims 27-30, wherein said cell has a reduced expression of a Type I or Type II IFN relative to a cell subjected to at least three consecutive rounds of contacting with exogenously introduced non-modified synthetic RNAs encoding the developmental potential altering factors OCT4, SOX2, KLF4 and c-MYC.

32. The cell of any one of claims 27-31, wherein said cell has a reduced expression of at least one IFN-signature gene relative to a human cell subjected to at least three consecutive rounds of contacting with exogenously introduced non-modified synthetic RNAs encoding the developmental potential altering factors OCT4, SOX2, KLF4 and c-MYC.

33. The cell of claim 32, wherein the IFN-signature gene is selected from the group consisting of IFNα, IFNB1, IFIT, OAS1, PKR, RIGI, CCL5, RAP1A, CXCL10, IFIT1, CXCL11, MX1, RP11-167P23.2, HERC5, GALR3, IFIT3, IFIT2, RSAD2, and CDC20.

34. The cell of any one of claims 27-33, wherein each of the synthetic, modified RNAs further comprises a 5' cap.

35. The cell of claim 34, wherein the 5' cap is a 5' cap analog.

36. The cell of claim 35, wherein the 5' cap analog is a 5' diguanosine cap.

37. The cell of any one of claims 27-36, wherein each of the synthetic, modified RNAs does not comprise a 5' triphosphate.

38. The cell of any one of claims 27-37, wherein each of the synthetic, modified RNAs further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof.

39. The cell of any one of claims 27-38, wherein each of the synthetic, modified RNAs is treated with an alkaline phosphatase.

40. The cell of any one of claims 27-39, wherein the cell or its immediate precursor cell(s) is derived from a somatic cell, partially reprogrammed somatic cell, an induced pluripotent stem cell, a multipotent cell, a differentiated cell, or an embryonic stem cell.

41. A composition comprising synthetic, modified RNAs encoding reprogramming factors OCT4, SOX2, KLF4, and c-MYC, and cell growth medium, wherein each cytosine of each of the synthetic, modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic, modified RNAs is replaced with pseudouridine.

42. The composition of claim 41, wherein said composition permits an efficiency of induced pluripotent stem cell generation from a starting population of somatic cells of at least 1%.

43. The composition of claims 41 or 42, wherein said composition permits a rate of induced pluripotent stem cell generation from a starting population of somatic cells of less than 25 days and greater than 7 days.

44. The composition of claim 43, wherein the composition further comprises a synthetic, modified RNA encoding LIN-28, wherein each cytosine of the synthetic, modified RNA encoding LIN-28 is replaced with 5-methylcytosine and each uracil of the synthetic, modified RNA encoding LIN-28 is replaced with pseudouridine.

45. The composition of any one of claims 41-44, wherein each of the synthetic, modified RNAs further comprises a 5' cap.

46. The composition of claim 45, wherein the 5' cap is a 5' cap analog.

47. The composition of claim 46, wherein the 5' cap analog is a 5' diguanosine cap.

48. The composition of any one of claims 41-47, wherein each of the synthetic, modified RNAs does not comprise a 5' triphosphate.

49. The composition of any one of claims 41-48, wherein each of the synthetic, modified RNAs further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof.

50. The composition of any one of claims 41-49, wherein each of the synthetic, modified RNAs is treated with a phosphatase.

51. A kit for reprogramming a somatic cell to an induced pluripotent stem cell, the kit comprising:

(a) a vial comprising a synthetic, modified RNA encoding an OCT4 reprogramming factor and a buffer;

(b) a vial comprising a synthetic, modified RNA encoding a SOX2 reprogramming factor and a buffer;

(c) a vial comprising a synthetic, modified RNA encoding a c-MYC reprogramming factor and a buffer;

(d) a vial comprising a synthetic, modified RNA encoding a KLF4 reprogramming factor and a buffer; and

(e) packaging and instructions therefor;

wherein each cytosine of each of the synthetic, modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic, modified RNAs is replaced with pseudouridine.

52. The kit of claim 51, wherein the concentration in the vial of each said synthetic, modified RNA encoding a reprogramming factor is 100 ng/mL.

53. The kit of claim 51 or 52, further comprising a vial comprising a synthetic, modified RNA molecule encoding a LIN28 reprogramming factor and a buffer, wherein each cytosine of the synthetic,

modified RNA encoding LIN-28 is replaced with 5-methylcytosine and each uracil of the synthetic, modified RNA encoding LIN-28 is replaced with pseudouridine.

54. The kit of any one of claims 51-53, wherein the buffer is RNase-free TE buffer at pH 7.0.

55. The kit of any one of claims 51-54, further comprising a synthetic, modified RNA encoding a positive control.

56. A kit for reprogramming a somatic cell to an induced pluripotent stem cell, the kit comprising:

(a) a container comprising a synthetic, modified RNA encoding an OCT4 reprogramming factor; a synthetic, modified RNA encoding a SOX2 reprogramming factor; a synthetic, modified RNA encoding a c-MYC reprogramming factor; a synthetic, modified RNA encoding a KLF4 reprogramming factor; and a buffer, wherein each cytosine of each of the synthetic, modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic, modified RNAs is replaced with pseudouridine; and

(b) packaging and instructions therefor.

57. The kit of claim 56, wherein the concentration in the container of the synthetic, modified RNAs encoding reprogramming factors is 100 ng/mL.

58. The kit of claim 56 or 57, further comprising a synthetic, modified RNA molecule encoding a LIN28 reprogramming factor, wherein each cytosine of the synthetic, modified RNA encoding LIN-28 is replaced with 5-methylcytosine and each uracil of the synthetic, modified RNA encoding LIN-28 is replaced with pseudouridine.

59. The kit of any one of claims 56-58, further comprising a synthetic, modified RNA encoding a positive control.

60. The kit any one of claims 56-59, wherein the buffer is RNase-free TE buffer at pH 7.0.

61. The kit of any one of claims 56-60, wherein each said synthetic, modified RNA encoding a reprogramming factor further comprises a ligand.

62. The kit of claim 61, wherein the ligand is a lipid or lipid-based molecule.

63. A kit for reprogramming a somatic cell to an induced pluripotent stem cell, the kit comprising:

(a) a vial comprising a synthetic, modified RNA encoding an OCT4 reprogramming factor and a buffer;

(b) a vial comprising a synthetic, modified RNA encoding an SOX2 reprogramming factor and a buffer;

(c) a vial comprising a synthetic, modified RNA encoding a c-MYC reprogramming factor and a buffer;

(d) a vial comprising a synthetic, modified RNA encoding a KLF4 reprogramming factor and a buffer;

(e) a vial comprising a synthetic, modified RNA molecule encoding a LIN28 reprogramming factor and a buffer;

(f) a vial comprising a synthetic, modified RNA encoding a positive control GFP molecule and a buffer; and packaging and instructions therefor;

wherein each cytosine of each of the synthetic, modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic, modified RNAs is replaced with pseudouridine, and wherein the buffers in each of said vials is an RNase-free buffer.

64. The kit of claim 63, wherein the RNase-free buffer is RNase-free TE buffer at pH 7.0.

65. The kit of claim 63 or 64, wherein the concentration of the synthetic, modified RNAs encoding OCT4, SOX2, c-MYC, KLF-4, LIN28 and GFP in each of the vials is 100 ng/mL.

66. Use of synthetic, modified RNAs encoding developmental potential altering factors OCT-4, SOX2, and c-MYC for contacting at least three times a cell or progeny cells thereof to reprogram the cell or progeny cells thereof into an induced pluripotent stem cell or induced pluripotent stem cell population, wherein each cytosine of each of the synthetic, modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic, modified RNAs is replaced with pseudouridine.

67. The use of claim 66, wherein each of the synthetic, modified RNAs further comprises a 5' cap.

68. The use of claim 67, wherein the 5' cap is a 5' cap analog.

69. The use of claim 68, wherein the 5' cap analog is a 5' diguanosine cap.

70. The use of any one of claims 66-69, wherein each of the synthetic, modified RNAs does not comprise a 5' triphosphate.

71. The use of any one of claims 66-70, wherein each of the synthetic, modified RNAs further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof.

72. The use of any one of claims 66-71, wherein the contacting of the cell or progeny cells thereof is performed *in vitro or ex vivo*.

73. Use of synthetic, modified RNAs encoding reprogramming factors OCT4, SOX2, KLF4, and c-MYC for contacting a somatic cell or progeny cells thereof at least five consecutive times to reprogram the somatic cell or progeny cells thereof into an induced pluripotent stem cell, wherein each cytosine of each of the synthetic, modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic, modified RNAs is replaced with pseudouridine.

74. The use of claim 73, wherein the at least five consecutive times occur within 25 days.

75. The use of claim 73 or 74, wherein each of the synthetic, modified RNA further comprises a 5' cap.

76. The use of claim 75, wherein the 5' cap is a 5' cap analog.

77. The use of claim 76, wherein the 5' cap analog is a 5' diguanosine cap.

78. The use of any one of claims 73-77, wherein each of the synthetic, modified RNAs does not comprise a 5' triphosphate.

79. The use of any one of claims 73-78, wherein each of the synthetic, modified RNAs further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof.

80. The use of any one of claims 73-79, wherein the somatic cell is a human somatic cell.

81. The use of claim 80, further comprising a synthetic, modified RNA molecule encoding LIN28, wherein each cytosine of each of the synthetic, modified RNA encoding LIN28 is replaced with 5-methylcytosine and each uracil of each of the synthetic, modified RNA encoding LIN28 is replaced with pseudouridine.

82. The use of any one of claims 73-81, wherein said contacting is under a low-oxygen condition.

83. The use of any one of claims 73-82, wherein said contacting of the somatic cell or progeny cells thereof is performed *in vitro or ex vivo*.

84. A composition for making induced pluripotent stem (iPS) cells comprising synthetic modified ribonucleic acids (RNAs) encoding OCT4, SOX2, and KLF4, and cell growth medium, wherein each cytosine of each of the synthetic modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic mRNAs is replaced with pseudouridine.

85. A kit comprising:

(a) a vial comprising a synthetic modified RNA encoding an OCT4 reprogramming factor and a buffer;

(b) a vial comprising a synthetic modified RNA encoding a SOX2 reprogramming factor and a buffer;

(c) a vial comprising a synthetic modified RNA encoding a KLF4 reprogramming factor and a buffer; and

(d) packaging and instructions therefor;

wherein each cytosine of each of the synthetic modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic modified RNAs is replaced with pseudouridine.

86. A method of reprogramming a somatic fibroblast cell into an induced pluripotent stem (iPS) cell, the method comprising contacting the somatic fibroblast cell with synthetic modified RNAs encoding the reprogramming factors OCT4, SOX2, and KLF4 at least five consecutive times within 25 days, wherein each cytosine of each of the synthetic modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic modified RNAs is replaced with pseudouridine, thereby producing an induced pluripotent stem (iPS) cell.

87. A kit for making induced pluripotent stem (iPS) cells from a fibroblast cell comprising the following components:

(a) a synthetic mRNA encoding OCT4, wherein each cytosine of the synthetic mRNA encoding OCT4 is replaced with 5-methylcytosine and each uracil is replaced with pseudouridine;

(b) a synthetic mRNA encoding SOX2, wherein each cytosine of the synthetic mRNA encoding SOX2 is replaced with 5-methylcytosine and each uracil is replaced with pseudouridine;

(c) a synthetic mRNA encoding KLF4, wherein each cytosine of the synthetic mRNA encoding KLF4 is replaced with 5-methylcytosine and each uracil is replaced with pseudouridine; and

(d) a synthetic mRNA encoding c-MYC, wherein each cytosine of the synthetic mRNA encoding c-MYC is replaced with 5-methylcytosine and each uracil is replaced with pseudouridine.

88. A method for making induced pluripotent stem (iPS) cells from a fibroblast cell comprising:

(a) subjecting the fibroblast cell in culture medium to a daily transfection with a transfection mixture comprising

(i) a synthetic mRNA mixture comprising a synthetic mRNA encoding OCT4; a synthetic mRNA encoding SOX2; a synthetic mRNA encoding KLF4; and a synthetic mRNA encoding c-MYC, wherein the synthetic mRNA encoding OCT4 is provided in molar excess of at least three times the concentration of the remaining three synthetic mRNAs, and wherein each cytosine of each of the synthetic mRNAs is replaced with 5-methylcytosine, and each uracil of each of the synthetic mRNAs is replaced with pseudouridine; and

(ii) a synthetic mRNA encoding a short half-life nuclear green fluorescent protein (GFP);

(b) providing culture conditions comprising 5% O2;

(c) removing the transfection mixture four hours after each daily transfection, from the cell in culture medium;

(d) culturing the cells in a culture medium not comprising the transfection mixture of step (a) for about three days to allow expansion of the cells;

(e) picking cells from the expanded cells of step (d), thereby generating iPS cells to generate iPS cell lines.

89. A kit for making synthetic mRNAs for the production of induced pluripotent stem (iPS) cells comprising the following components:

(a) a linear DNA template for the generation of a synthetic modified RNA, the template encoding an OCT4 mRNA of SEQ ID NO: 788;

(b) a linear DNA template for the generation of a synthetic modified RNA, the template encoding a SOX2 mRNA of SEQ ID NO: 941 or SEQ ID NO: 1501;

(c) a linear DNA template for the generation of a synthetic modified RNA, the template encoding a KLF4 mRNA of SEQ ID NO: 501;

(d) a linear DNA template for the generation of a synthetic modified RNA, the template encoding a c-MYC mRNA of SEQ ID NO: 636; and

(e) a ribonucleoside mixture component comprising 5-methylcytidine and pseudouridine.

90. An isolated, human induced pluripotent stem (iPS) cell comprising a transfection mixture comprising synthetic modified RNAs encoding octamer-binding transcription factor 4 (Oct4), sex determining region Y-box 2 (Sox2), and Kruppel-like factor 4 (Klf4), wherein each occurrence of a cytosine is replaced

with 5-methylcytosine (5mC) and each occurrence of a uracil is replaced with pseudouridine; wherein the iPS cell is produced by contacting an isolated human somatic fibroblast cell with the transfection mixture for a time sufficient to obtain the iPS cell.

91. A method of making induced pluripotent stem cells, the method comprising contacting mammalian somatic cells in vitro with synthetic, modified RNAs encoding OCT4, SOX2, and KLF4 at least three times, wherein each cytosine of each of the synthetic modified RNAs is replaced with 5-methylcytosine and each uracil of each of the synthetic modified RNAs is replaced with pseudouridine, such that iPS cells are obtained.

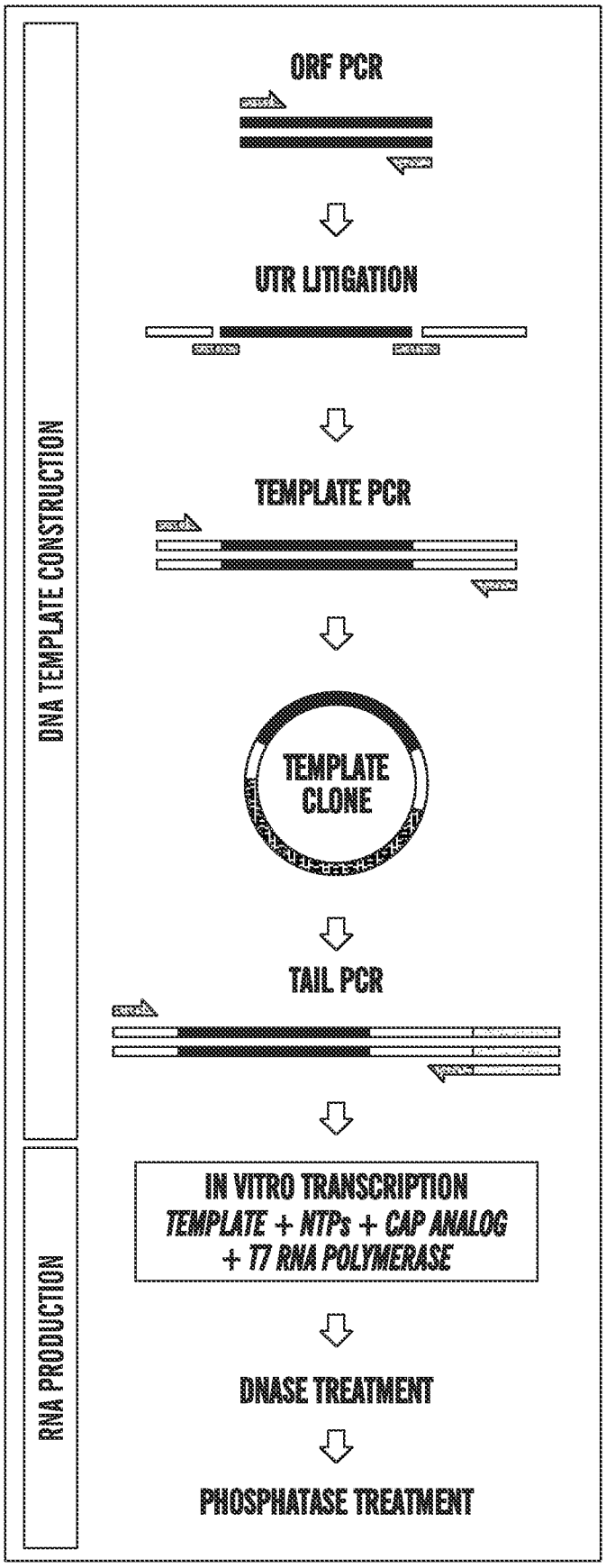

DNA TEMPLATE CONSTRUCTION
RNA PRODUCTION
ORF PCR
UTR LITIGATION
TEMPLATE PCR
TEMPLATE CLONE
TAIL PCR
IN VITRO TRANSCRIPTION
TEMPLATE + NTPs + CAP ANALOG
+ T7 RNA POLYMERASE
DNASE TREATMENT
PHOSPHATASE TREATMENT

FIG. 1

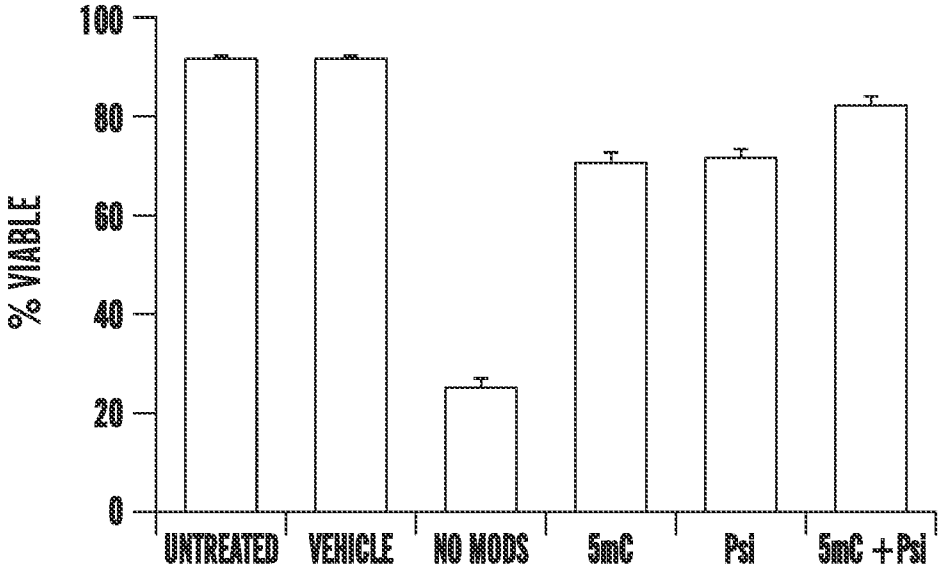

% VIABLE
100
80
60
40
20
0
UNTREATED
VEHICLE
NO MODS
5mC
Psi
5mC +Psi

FIG. 2A

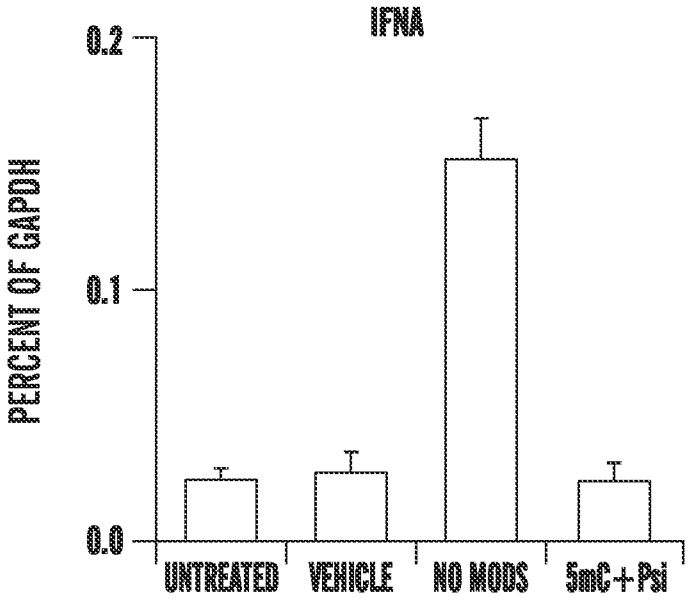

IFNA
PERCENT OF GAPDH
0.2
0.1
0.0
UNTREATED
VEHICLE
NO MODS
5mC+Psi

FIG. 2B

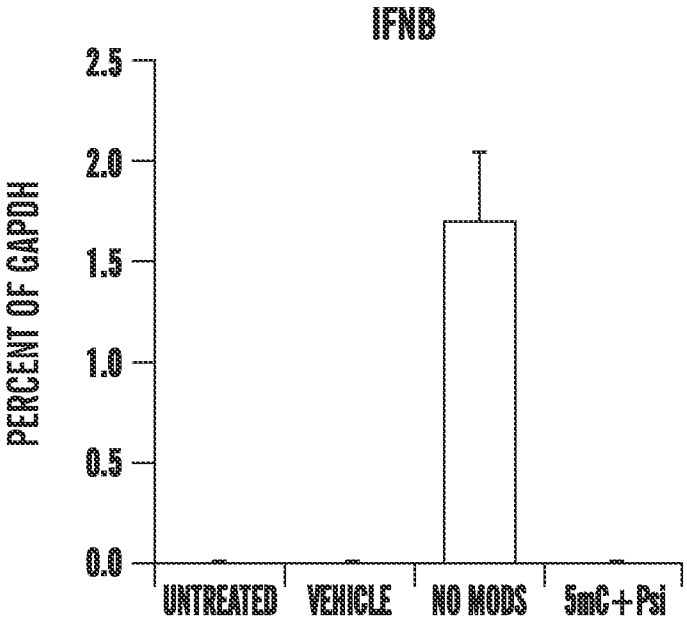

IFNB
PERCENT OF GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
UNTREATED
VEHICLE
NO MODS
5mC+Psi

FIG. 2C

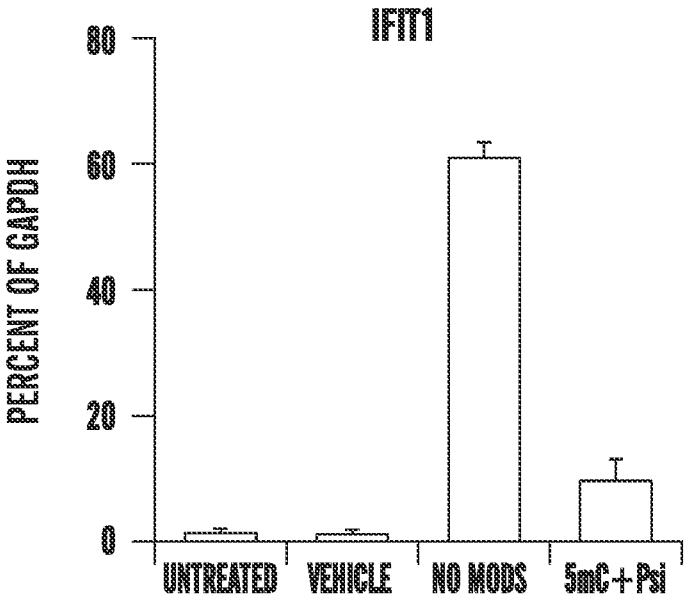

IFIT1
PERCENT OF GAPDH
80
60
40
20
0
UNTREATED
VEHICLE
NO MODS
5mC+Psi

FIG. 2D

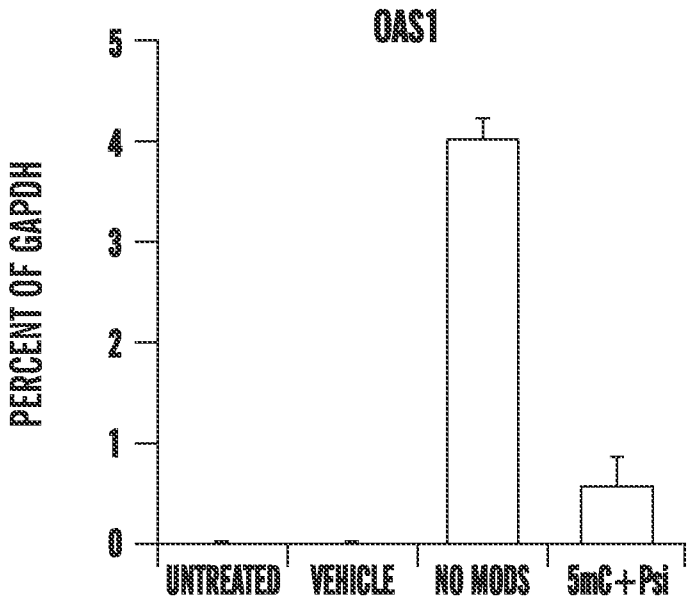

OAS1
PERCENT OF GAPDH
5
4
3
2
1
0
UNTREATED
VEHICLE
NO MODS
5mC+Psi

FIG. 2E

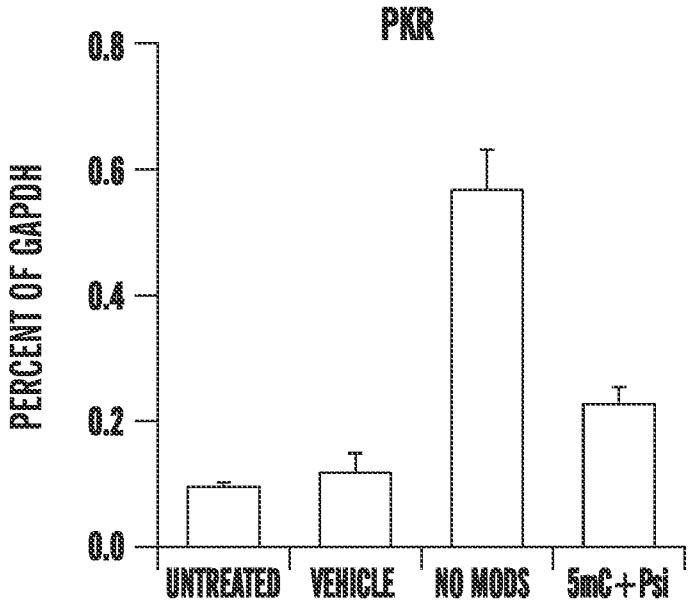

PKR
PERCENT OF GAPDH
0.8
0.6
0.4
0.2
0.0
UNTREATED
VEHICLE
NO MODS
5mC+Psi

FIG. 2F

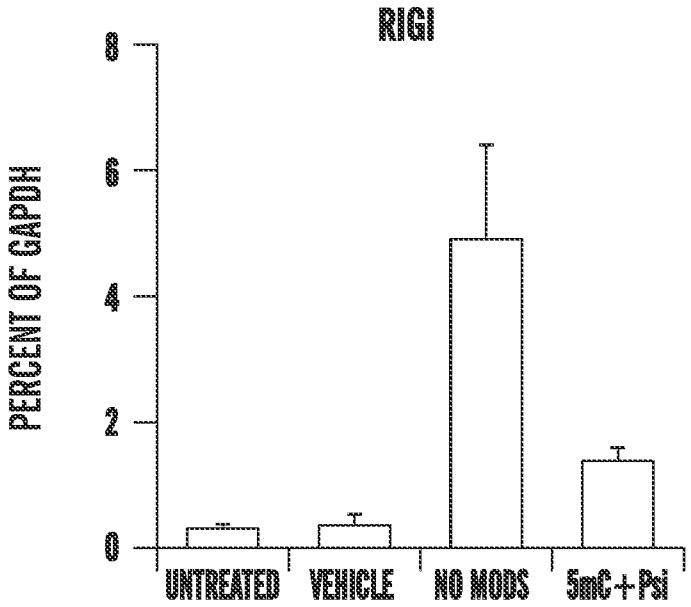

RIGI
PERCENT OF GAPDH
8
6
4
2
0
UNTREATED
VEHICLE
NO MODS
5mC+Psi

FIG. 2G

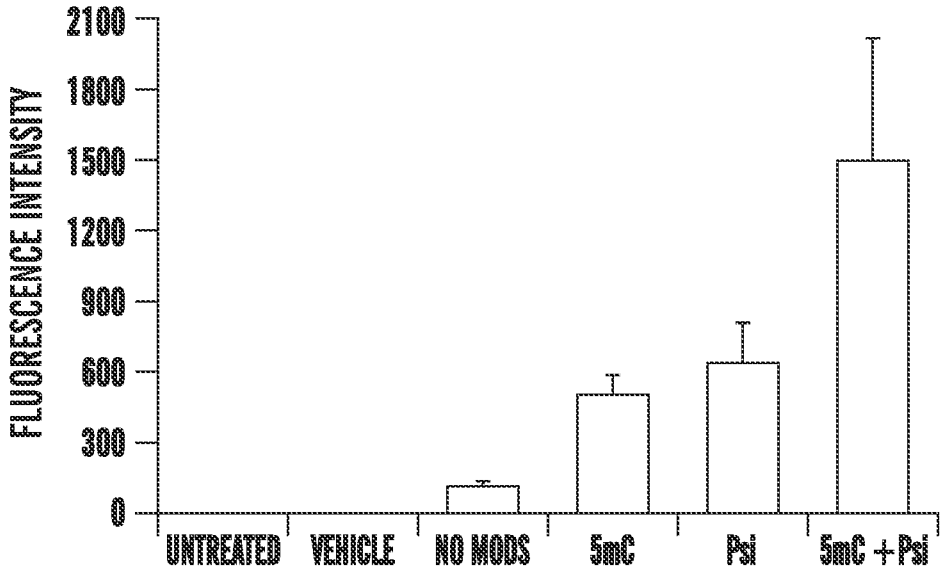

FLUORESCENCE INTENSITY
2100
1800
1500
1200
900
600
300
0
UNTREATED
VEHICLE
NO MODS
5mC
Psi
5mC +Psi

FIG. 2H

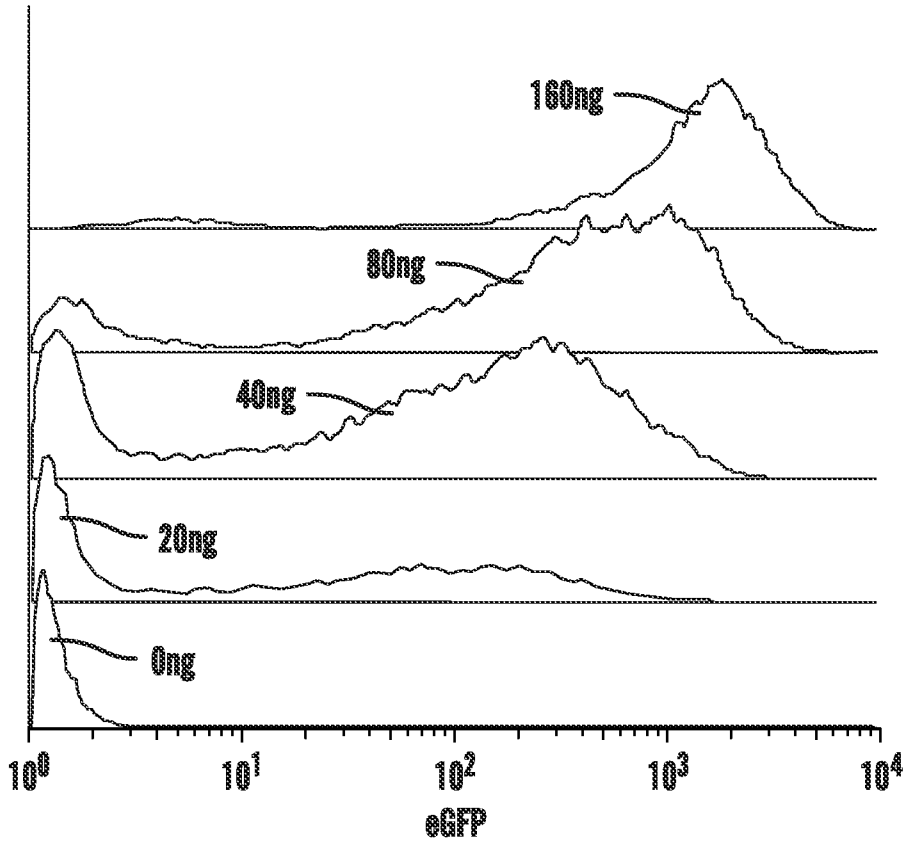

160ng
80ng
40ng
20ng
0ng
10^0
10^1
10^2
10^3
10^4
eGFP

FIG. 2I

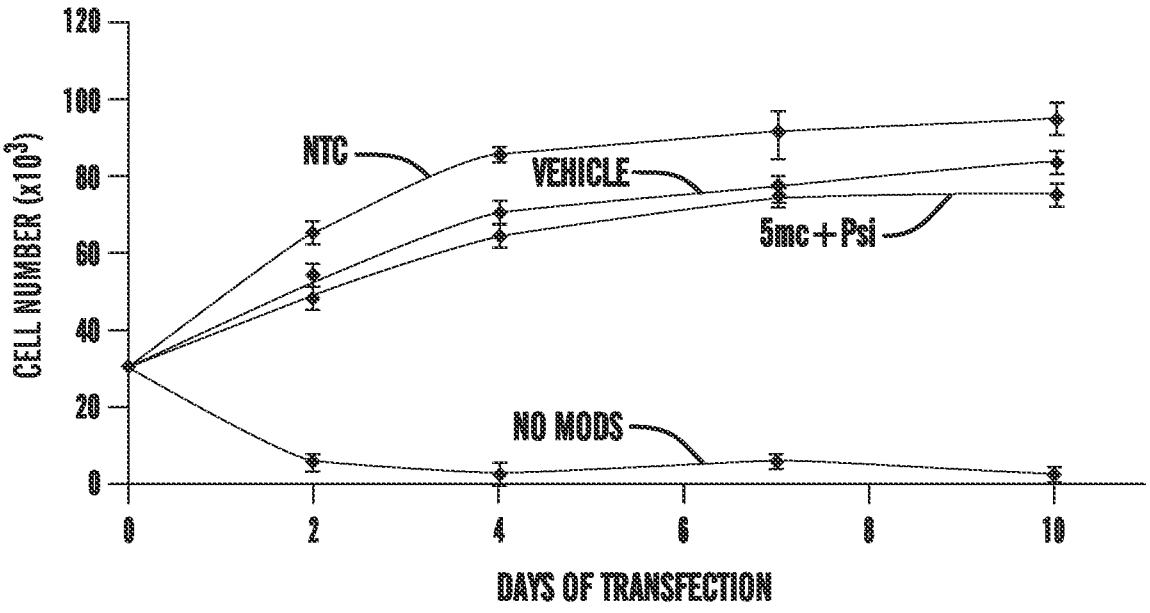

120
100
80
60
40
20
0
CELL NUMBER (x10^3)
NTC
VEHICLE
5mc + Psi
NO MODS
0
2
4
6
8
10
DAYS OF TRANSFECTION

FIG. 2J

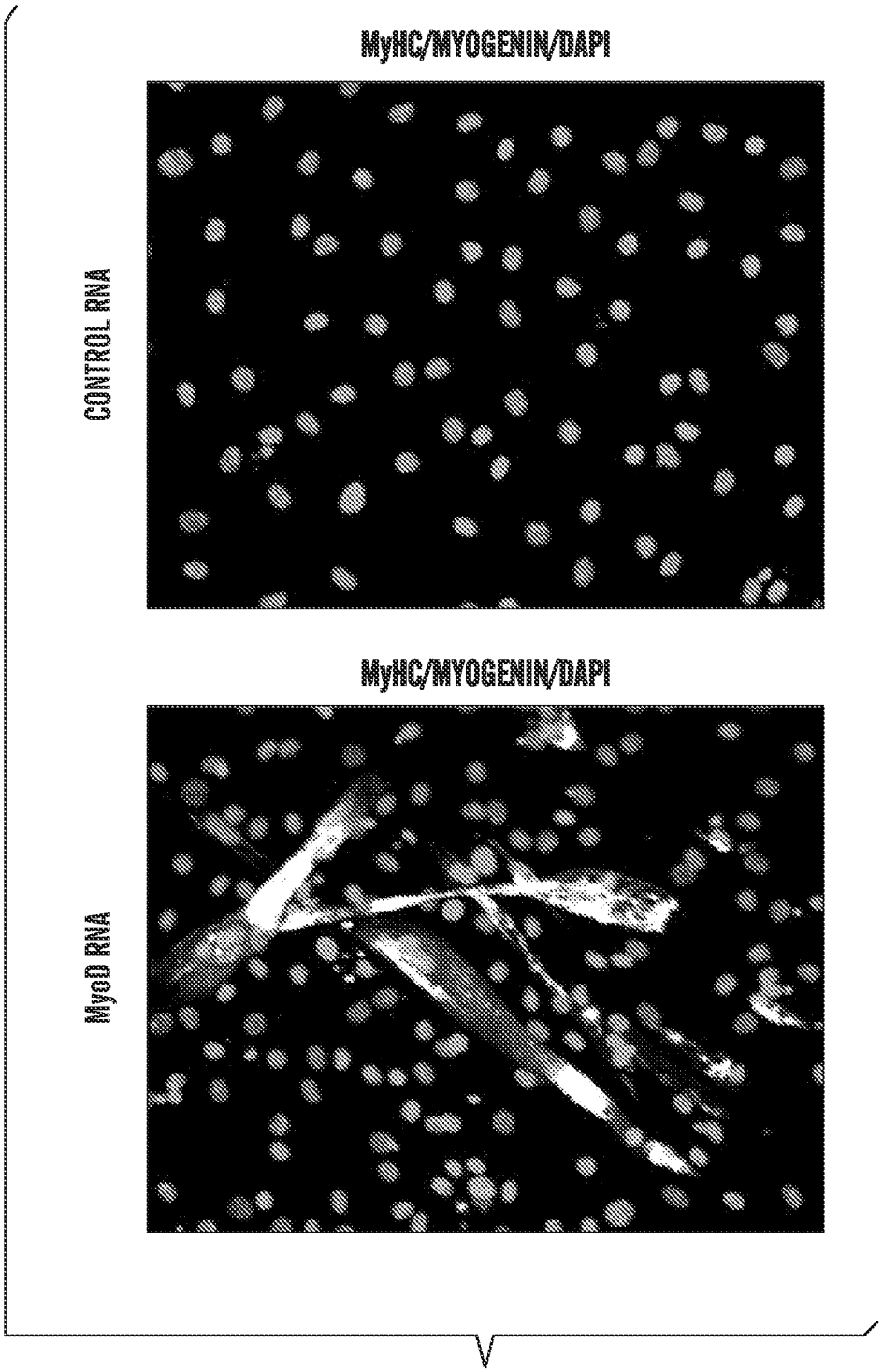


FIG. 2K

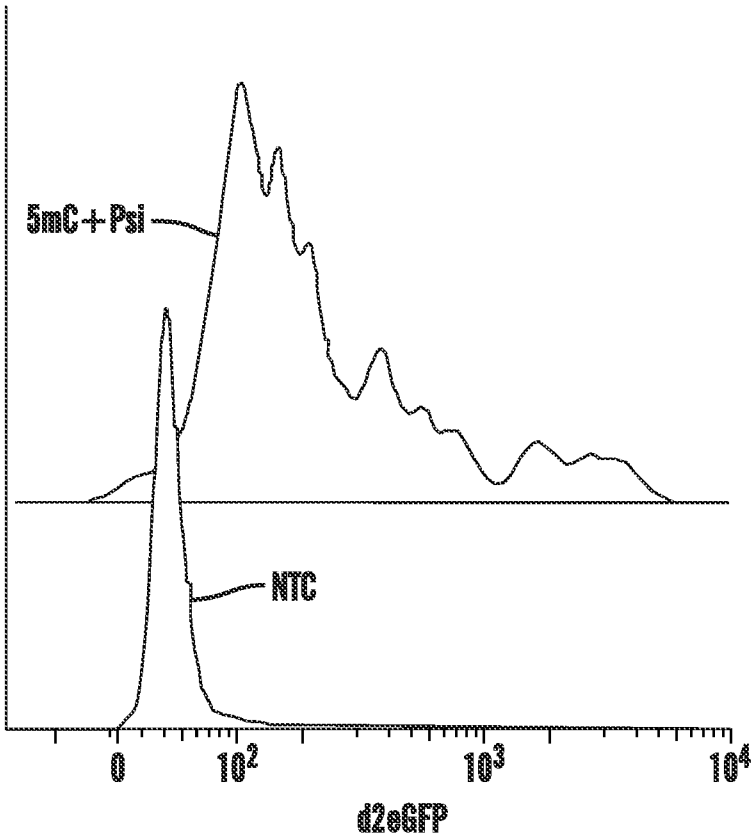


FIG. 2L

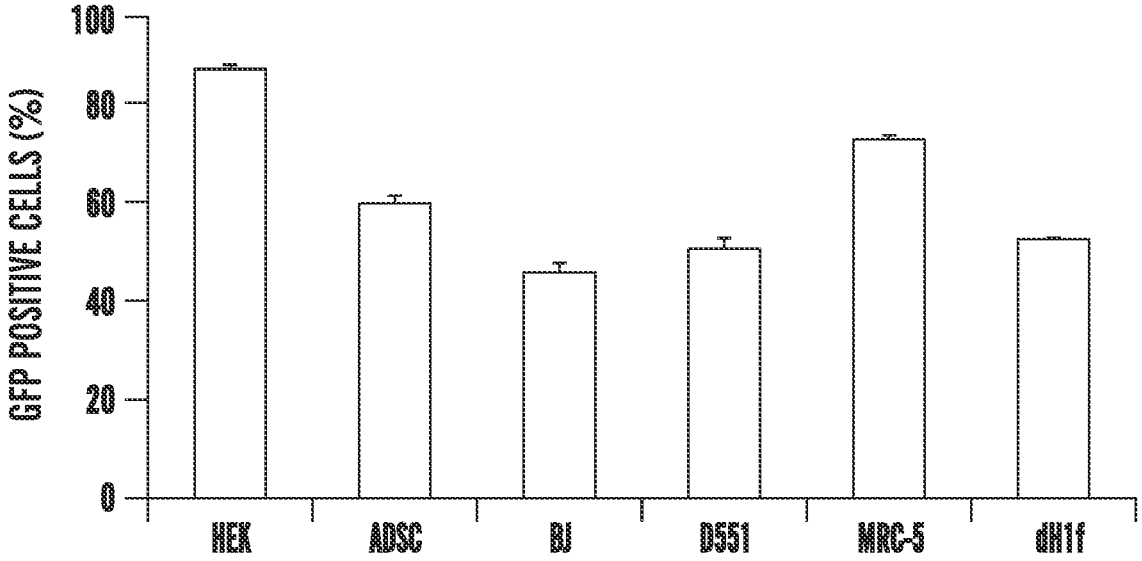

GFP POSITIVE CELLS (%)
100
80
60
40
20
0
HEK
ADSC
BJ
D551
MRC-5
dH1f

FIG. 3A

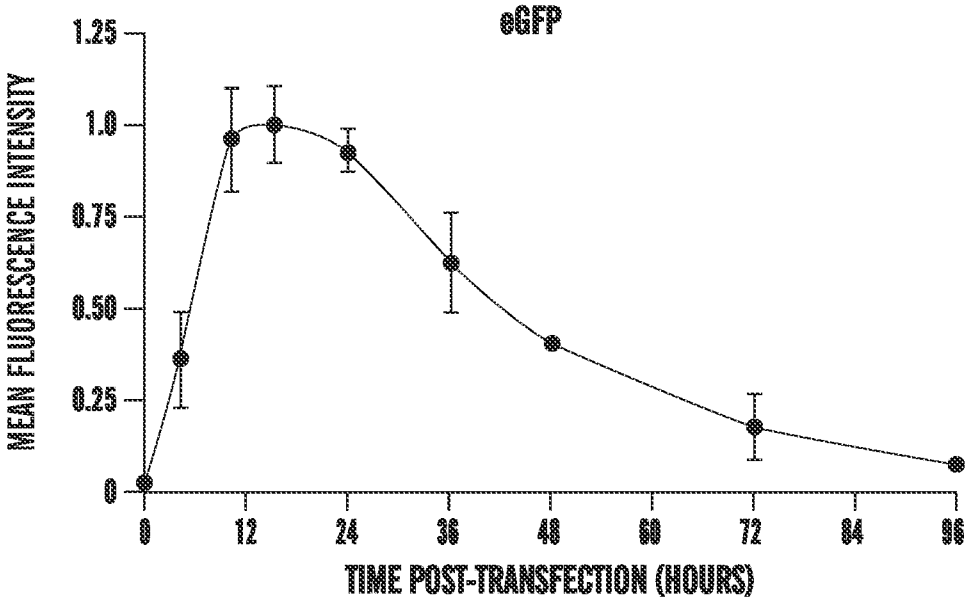

eGFP
MEAN FLUORESCENCE INTENSITY
1.25
1.0
0.75
0.50
0.25
0
0
12
24
36
48
60
72
84
96
TIME POST-TRANSFECTION (HOURS)

FIG. 3B

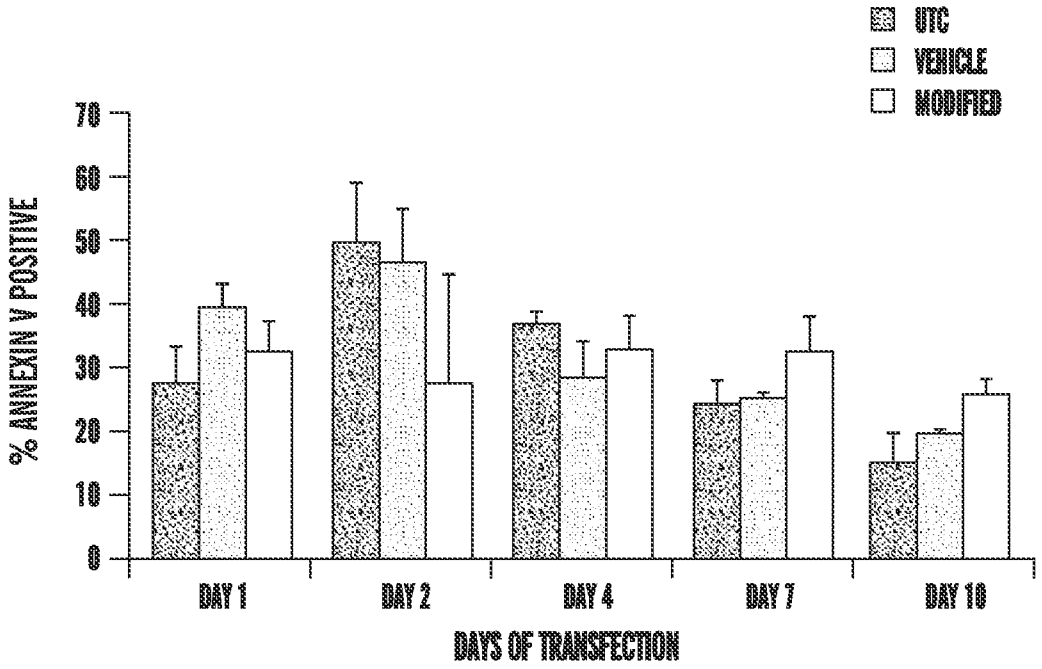

UTC
VEHICLE
MODIFIED
% ANNEXIN V POSITIVE
70
60
50
40
30
20
10
0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 10
DAYS OF TRANSFECTION

FIG. 3C

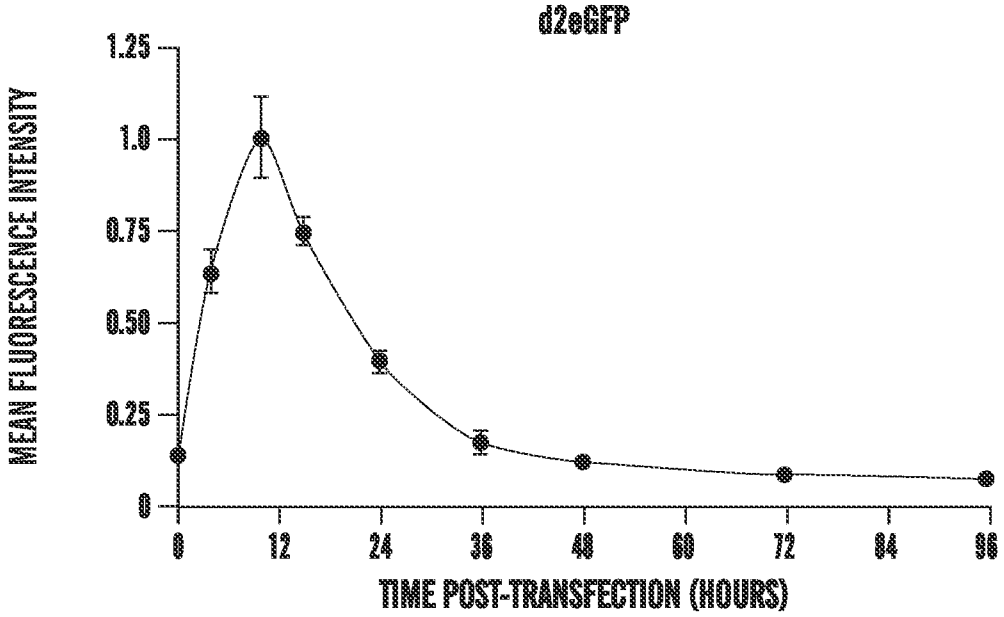

d2eGFP
MEAN FLUORESCENCE INTENSITY
1.25
1.0
0.75
0.50
0.25
0
0
12
24
36
48
60
72
84
96
TIME POST-TRANSFECTION (HOURS)

FIG. 3D

> 2-FOLD UPREGULATED IN MODIFIED-RNA VERSUS

UNTRANSFECTED		VEHICLE	
GENE	FOLD CHANGE	GENE	FOLD CHANGE
RAP1A	5.9	CCL5	4.4
IFIT1	5.1	RAP1A	4.4
CCL5	4.6	CXCL10	4.2
CXCL 10	4.4	IFIT1	4.0
CXCL 11	3.6	CXCL 11	3.4
RP11-167P23.2	3.5	MX1	3.2
GALR3	3.3	RP11-167P23.2	2.8
MX1	3.2	HERC5	2.8
HERC5	2.7	GALR3	2.6
IFIT2	2.6	IFIT3	2.5
RSAD2	2.6	IFIT2	2.5
CDC20	2.5	RSAD2	2.4
IFIT3	2.5	IFNB1	2.3
OASL	2.4	OASL	2.3
IFNB1	2.2	CDC20	2.0
CLDN1	2.2		
DKK1	2.1		
CMPK2	2.0		
DLL4	2.0		

FIG. 3E

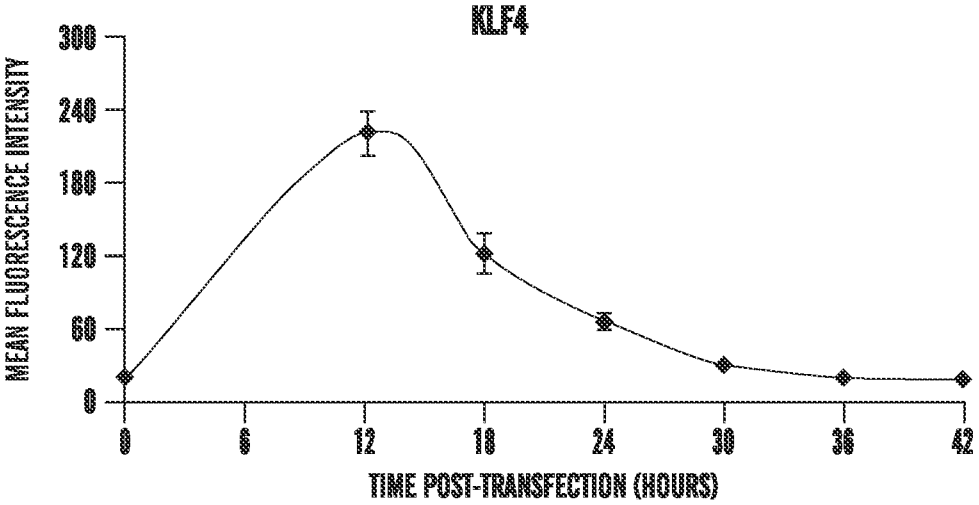


FIG. 4A

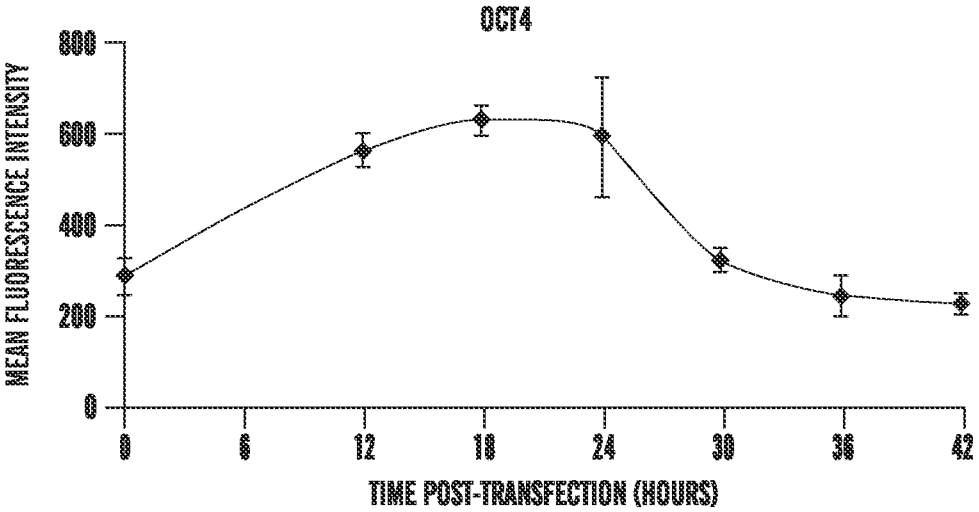


FIG. 4B

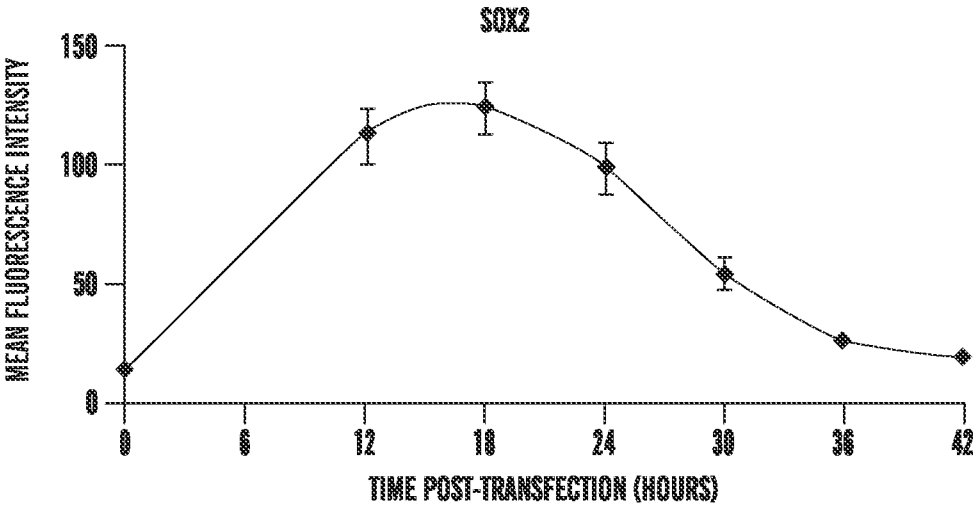


FIG. 4C

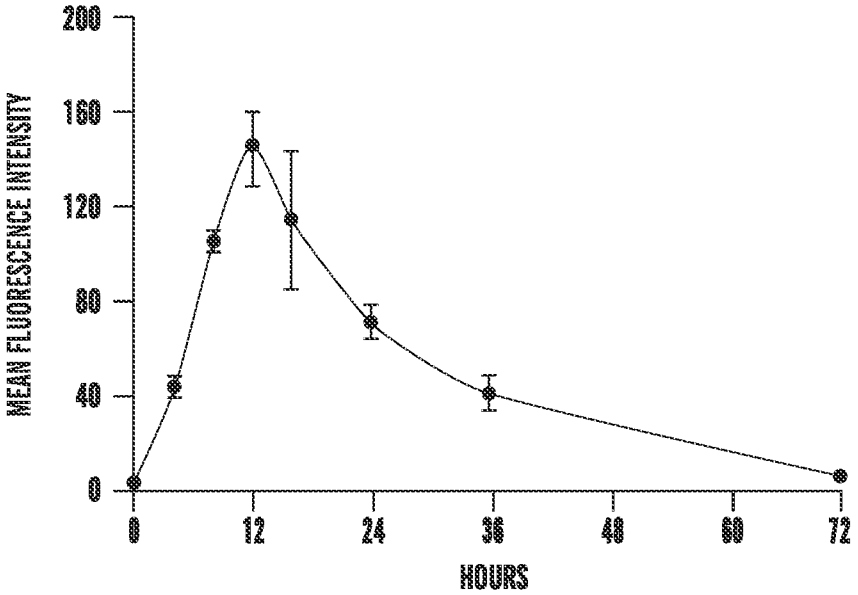

MEAN FLUORESCENCE INTENSITY
200
160
120
80
40
0
0
12
24
36
48
60
72
HOURS

FIG. 5

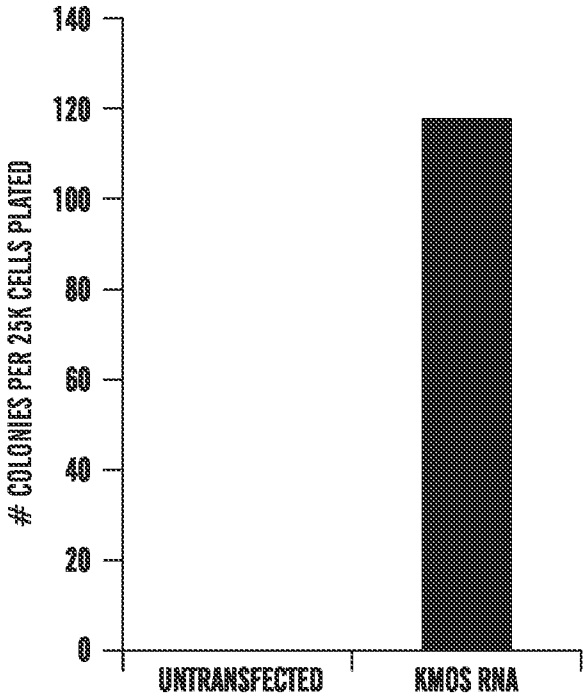

COLONIES PER 25K CELLS PLATED
140
120
100
80
60
40
20
0
UNTRANSFECTED
KMOS RNA

FIG. 6

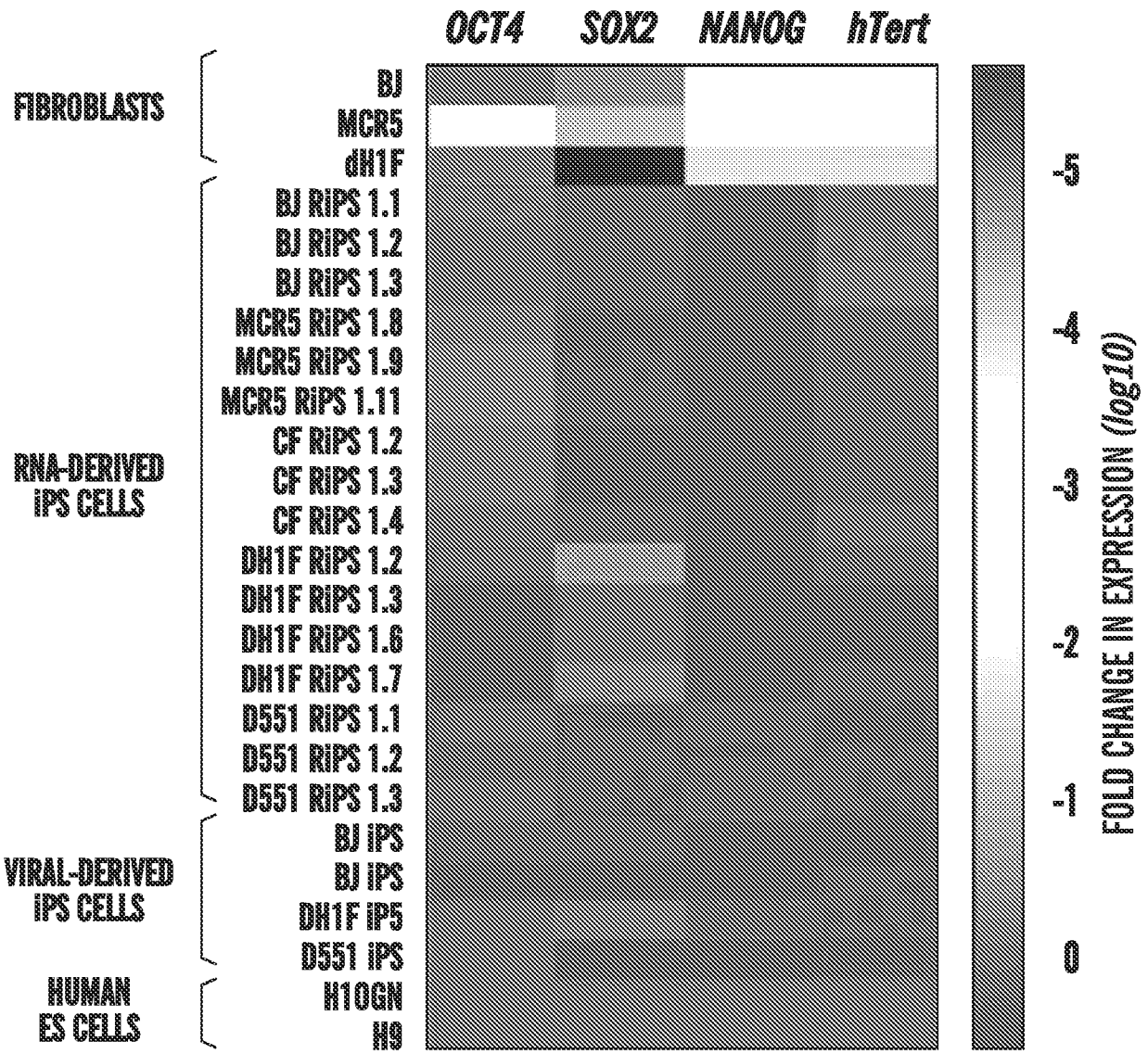

OCT4
SOX2
NANOG
hTert
FIBROBLASTS
BJ
MCR5
dH1F
RNA-DERIVED iPS CELLS
BJ RiPS 1.1
BJ RiPS 1.2
BJ RiPS 1.3
MCR5 RiPS 1.8
MCR5 RiPS 1.9
MCR5 RiPS 1.11
CF RiPS 1.2
CF RiPS 1.3
CF RiPS 1.4
DH1F RiPS 1.2
DH1F RiPS 1.3
DH1F RiPS 1.6
DH1F RiPS 1.7
D551 RiPS 1.1
D551 RiPS 1.2
D551 RiPS 1.3
VIRAL-DERIVED iPS CELLS
BJ iPS
BJ iPS
DH1F iP5
D551 iPS
HUMAN ES CELLS
H10GN
H9
5
4
3
2
1
0
FOLD CHANGE IN EXPRESSION (log10)

FIG. 7A

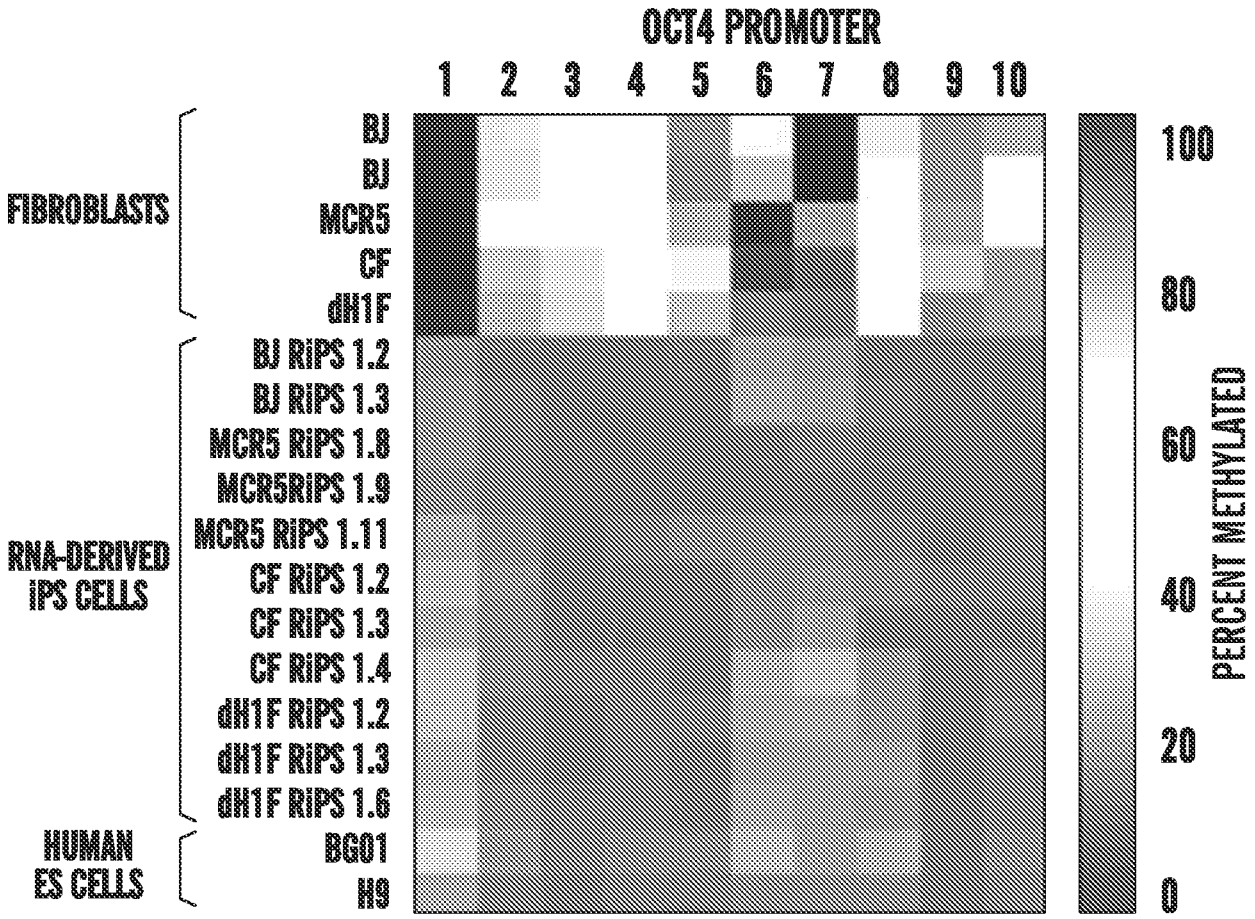

OCT4 PROMOTER
1 2 3 4 5 6 7 8 9 10
FIBROBLASTS
BJ
BJ
MCR5
CF
dH1F
RNA-DERIVED iPS CELLS
BJ RIPS 1.2
BJ RIPS 1.3
MCR5 RIPS 1.8
MCR5RIPS 1.9
MCR5 RIPS 1.11
CF RIPS 1.2
CF RIPS 1.3
CF RIPS 1.4
dH1F RIPS 1.2
dH1F RIPS 1.3
dH1F RIPS 1.6
HUMAN ES CELLS
BG01
H9
100
80
60
40
20
0
PERCENT METHYLATED

FIG. 7B

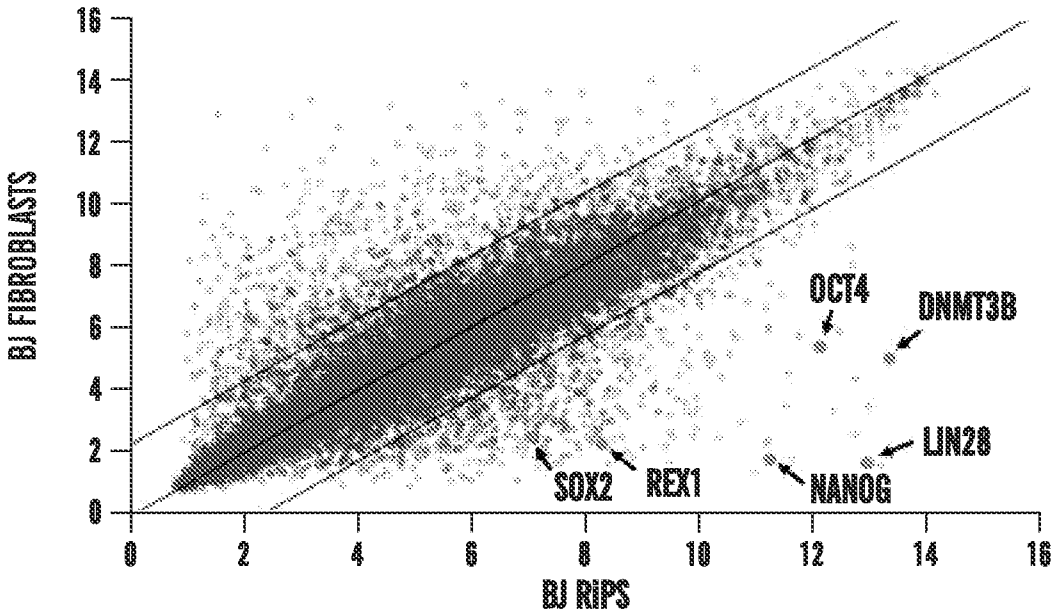

BJ FIBROBLASTS
BJ RIPS
OCT4
DNMT3B
LIN28
SOX2
REX1
NANOG
0
2
4
6
8
10
12
14
16

FIG. 7C

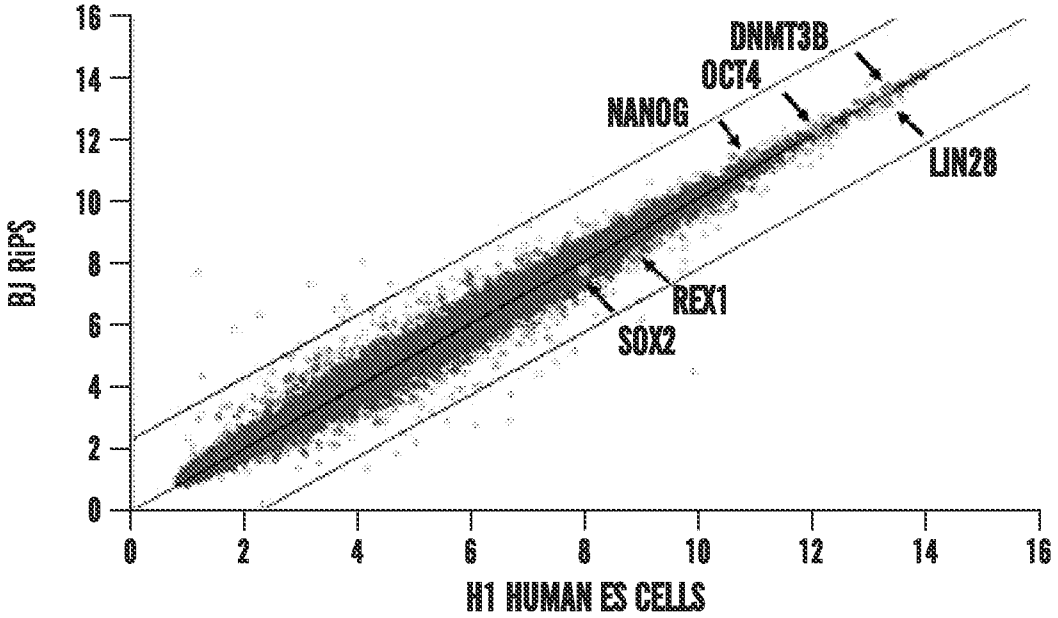

BJ RIPS
H1 HUMAN ES CELLS
DNMT3B
OCT4
NANOG
LIN28
REX1
SOX2
0
2
4
6
8
10
12
14
16

FIG. 7D

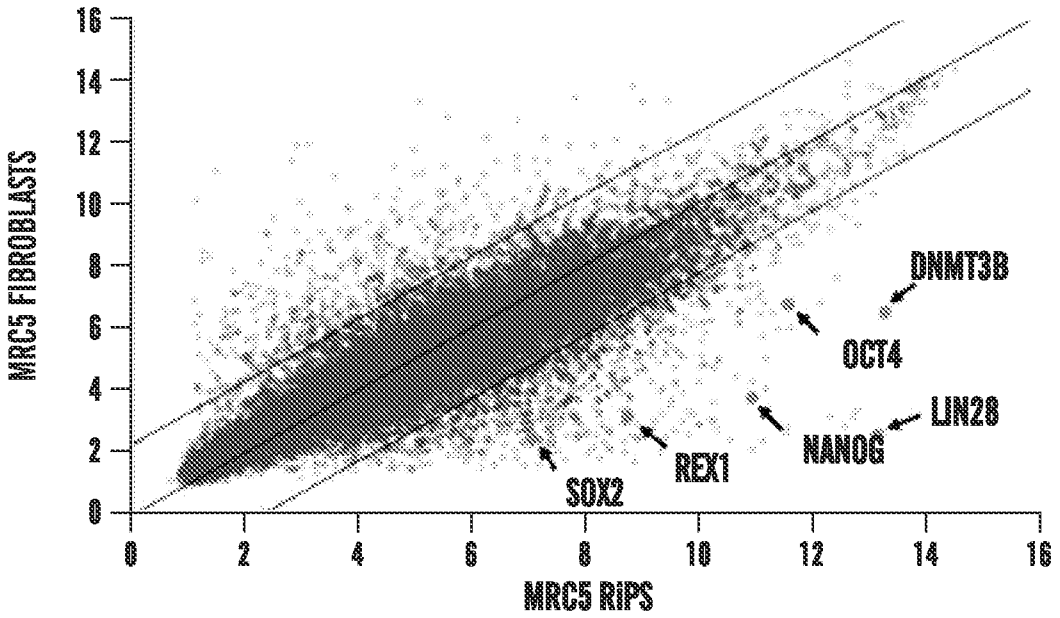

MRC5 FIBROBLASTS
MRC5 RiPS
0
2
4
6
8
10
12
14
16
DNMT3B
OCT4
LIN28
NANOG
REX1
SOX2

FIG. 7E

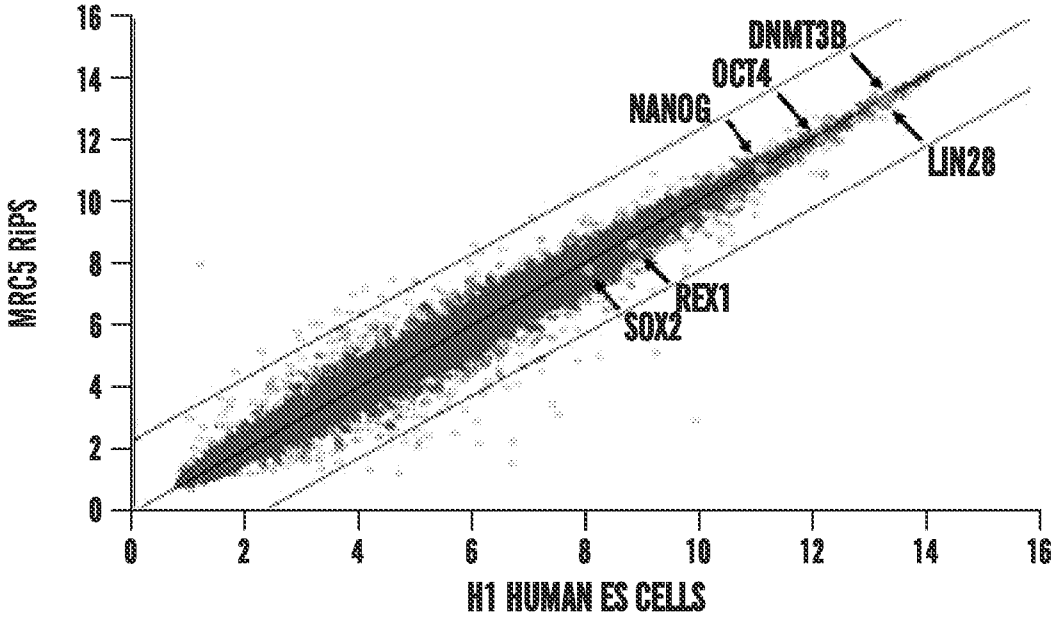

MRC5 RiPS
H1 HUMAN ES CELLS
0
2
4
6
8
10
12
14
16
DNMT3B
OCT4
NANOG
LIN28
REX1
SOX2

FIG. 7F

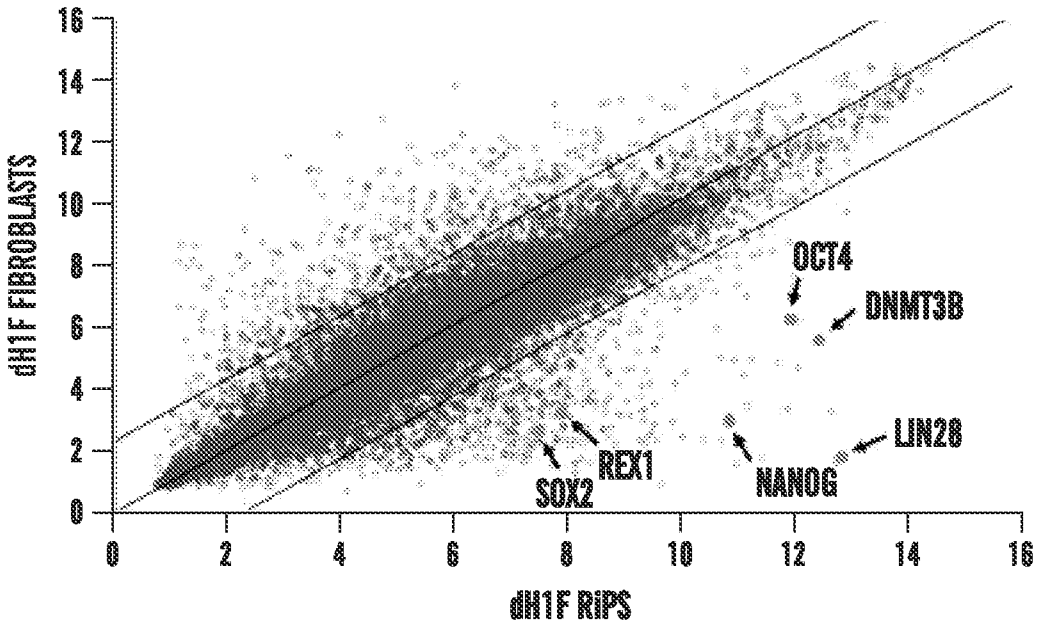

dH1F FIBROBLASTS
dH1F RiPS
OCT4
DNMT3B
LIN28
REX1
SOX2
NANOG
0
2
4
6
8
10
12
14
16

FIG. 7G

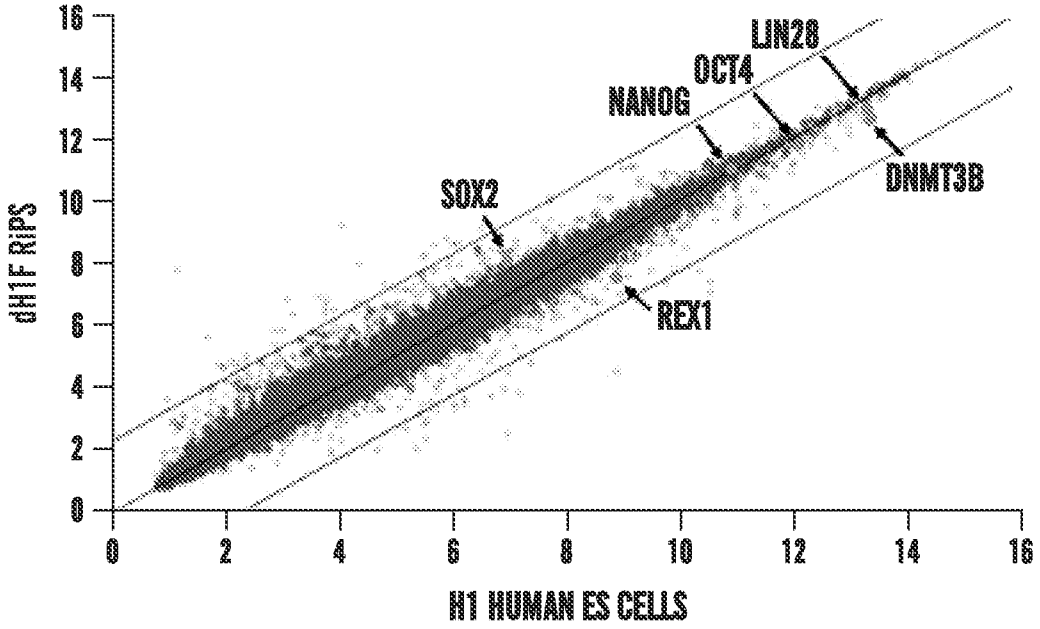

dH1F RiPS
H1 HUMAN ES CELLS
LIN28
OCT4
NANOG
SOX2
REX1
DNMT3B
0
2
4
6
8
10
12
14
16

FIG. 7H

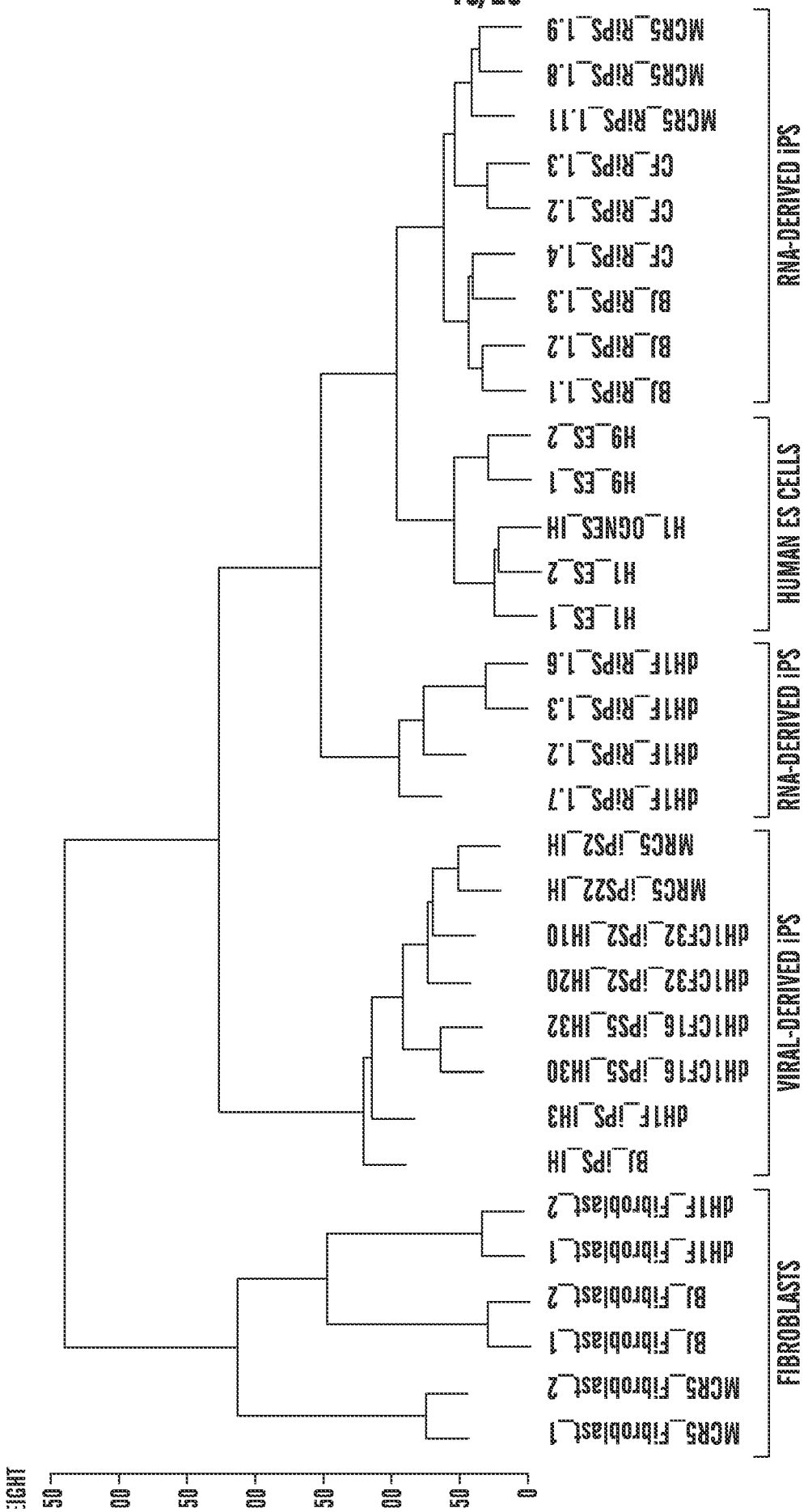

MCRS_RIPS_1.9
MCRS_RIPS_1.8
MCRS_RIPS_1.11
CF_RIPS_1.3
CF_RIPS_1.2
CF_RIPS_1.4
BJ_RIPS_1.3
BJ_RIPS_1.2
BJ_RIPS_1.1
RNA-DERIVED iPS
H9_ES_2
H9_ES_1
H1_OGNES_IH
H1_ES_2
H1_ES_1
HUMAN ES CELLS
dH1F_RIPS_1.6
dH1F_RIPS_1.3
dH1F_RIPS_1.2
dH1F_RIPS_1.7
RNA-DERIVED iPS
MRC5_iPS2_IH
MRC5_iPS22_IH
dH1CF32_iPS2_IH10
dH1CF32_iPS2_IH20
dH1CF16_iPS5_IH32
dH1CF16_iPS5_IH30
dH1F_iPS_IH3
BJ_iPS_IH
VIRAL-DERIVED iPS
dH1F_Fibroblast_2
dH1F_Fibroblast_1
BJ_Fibroblast_2
BJ_Fibroblast_1
MCRS_Fibroblast_2
MCRS_Fibroblast_1
FIBROBLASTS
50
00
50
00
50
00
50
0

FIG. 7I

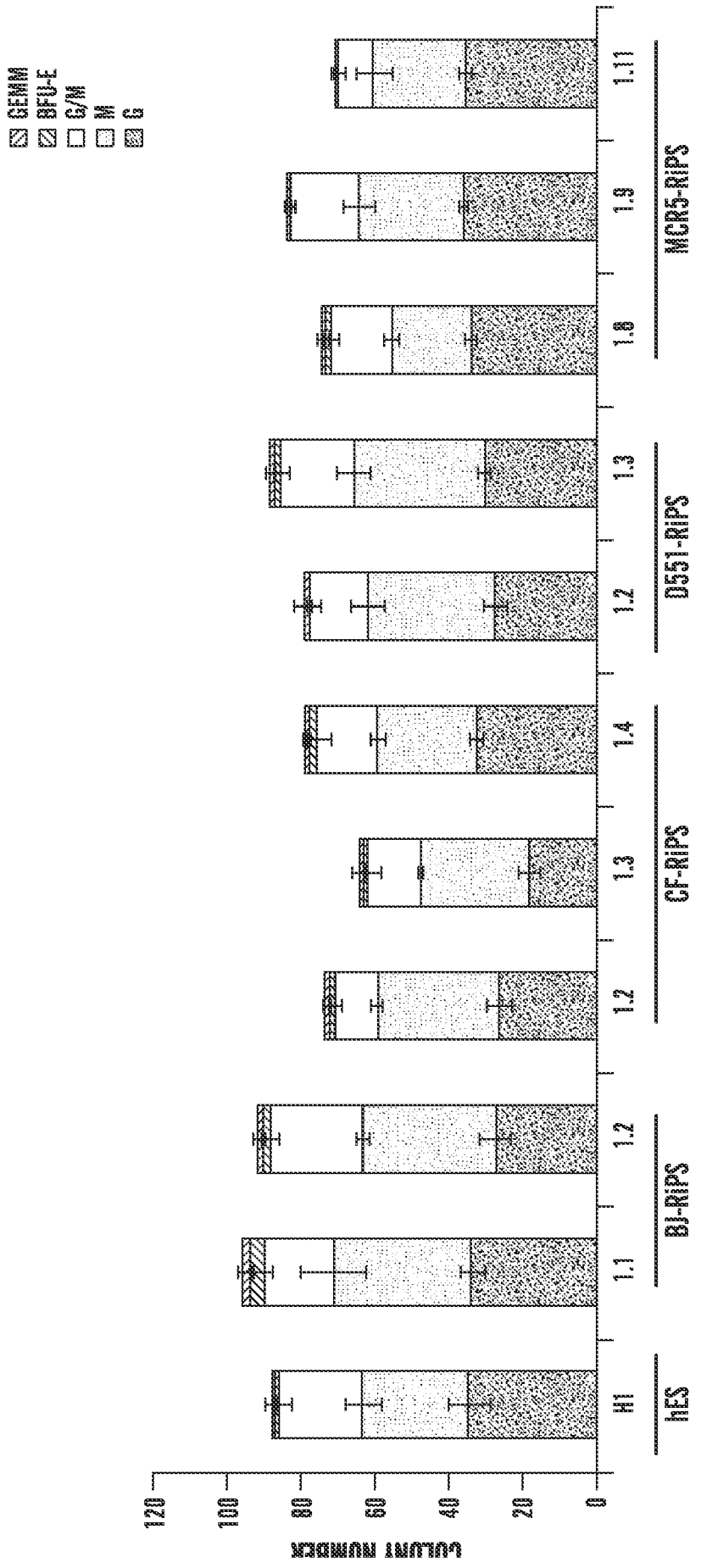

GEMM
BFU-E
G/M
M
G
COLONY NUMBER
120
100
80
60
40
20
0
H1
1.1
1.2
1.2
1.3
1.4
1.2
1.3
1.8
1.9
1.11
hES
BJ-RIPS
CF-RIPS
D551-RIPS
MCR5-RIPS

FIG. 8

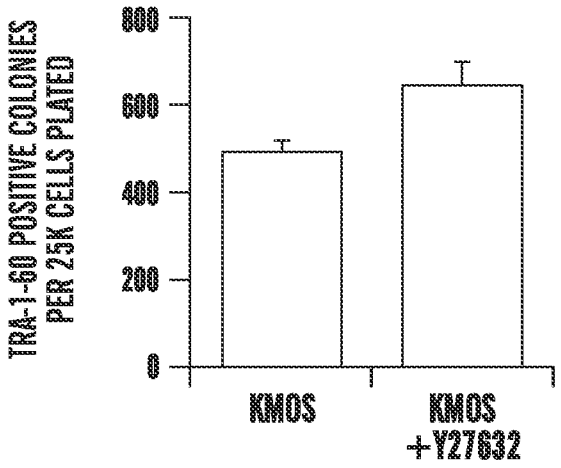

TRA-1-60 POSITIVE COLONIES PER 25K CELLS PLATED
800
600
400
200
0
KMOS
KMOS +Y27632

FIG. 9A

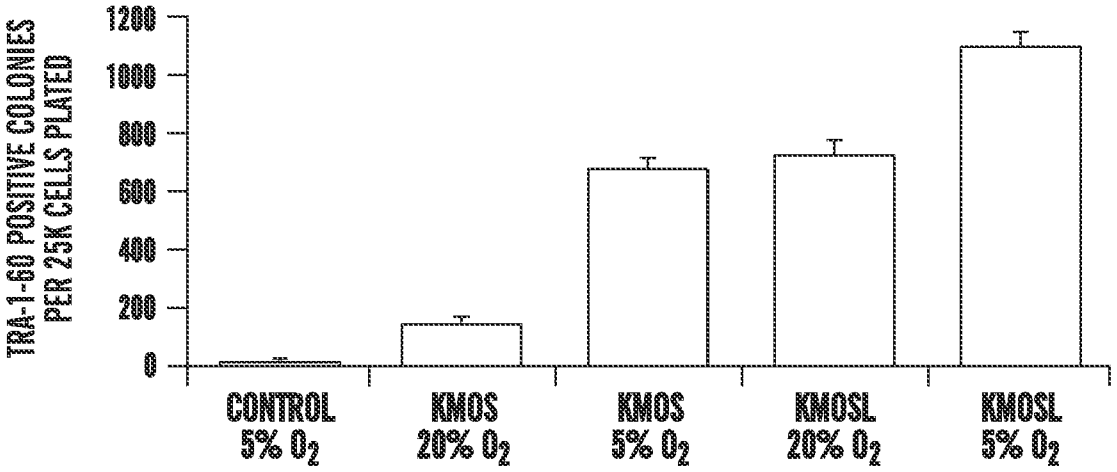

TRA-1-60 POSITIVE COLONIES PER 25K CELLS PLATED
1200
1000
800
600
400
200
0
CONTROL 5% O_2
KMOS 20% O_2
KMOS 5% O_2
KMOSL 20% O_2
KMOSL 5% O_2

FIG. 9B

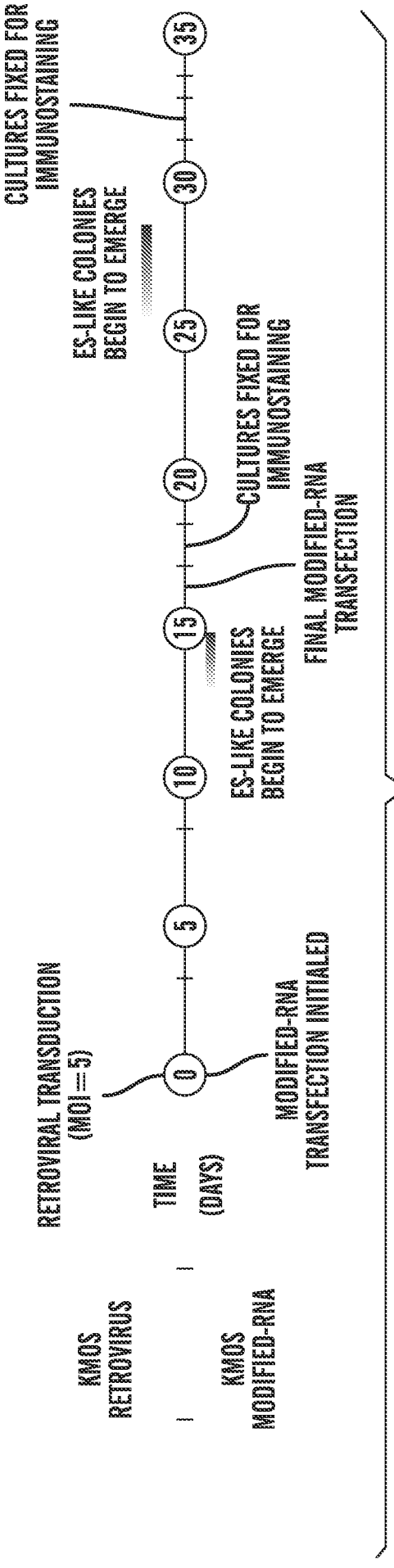

KMOS RETROVIRUS
KMOS MODIFIED-RNA
TIME (DAYS)
0
5
10
15
20
25
30
35
RETROVIRAL TRANSDUCTION (MOI=5)
MODIFIED-RNA TRANSFECTION INITIALED
ES-LIKE COLONIES BEGIN TO EMERGE
CULTURES FIXED FOR IMMUNOSTAINING
ES-LIKE COLONIES BEGIN TO EMERGE
FINAL MODIFIED-RNA TRANSFECTION
CULTURES FIXED FOR IMMUNOSTAINING

FIG. 9C

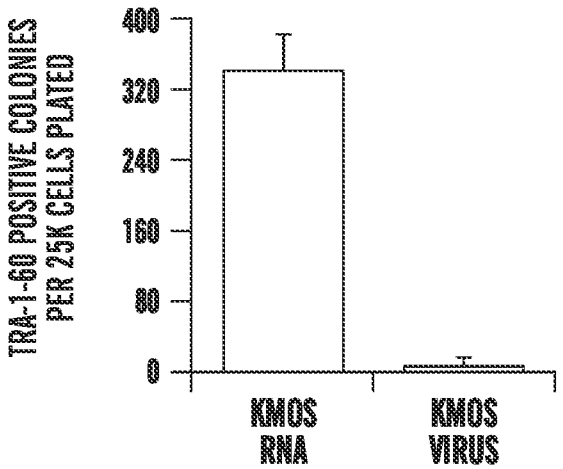

TRA-1-60 POSITIVE COLONIES PER 25K CELLS PLATED
400
320
240
160
80
0
KMOS RNA
KMOS VIRUS

FIG. 9D

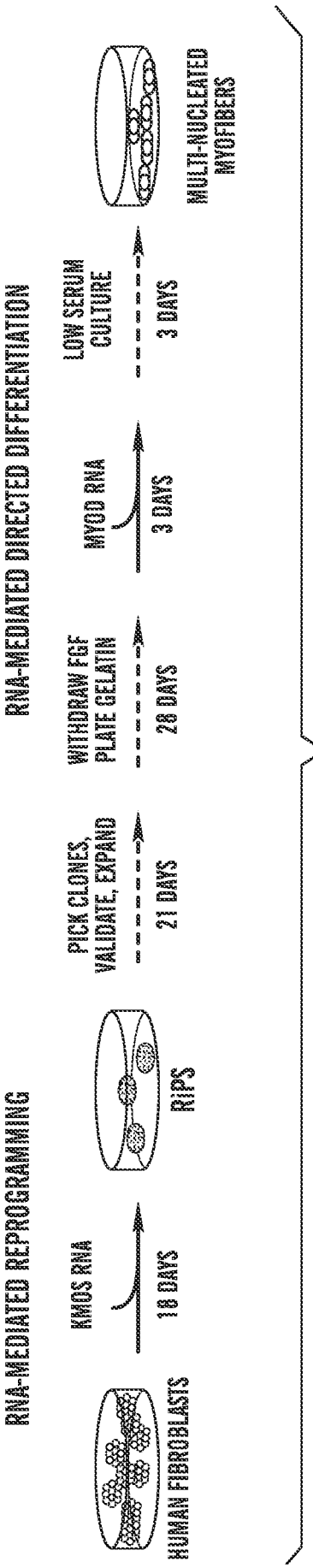

RNA-MEDIATED REPROGRAMMING
RNA-MEDIATED DIRECTED DIFFERENTIATION
HUMAN FIBROBLASTS
KMOS RNA
18 DAYS
RIPS
PICK CLONES, VALIDATE, EXPAND
21 DAYS
WITHDRAW FGF PLATE GELATIN
28 DAYS
MYOD RNA
3 DAYS
LOW SERUM CULTURE
3 DAYS
MULTI-NUCLEATED MYOFIBERS

FIG. 10A

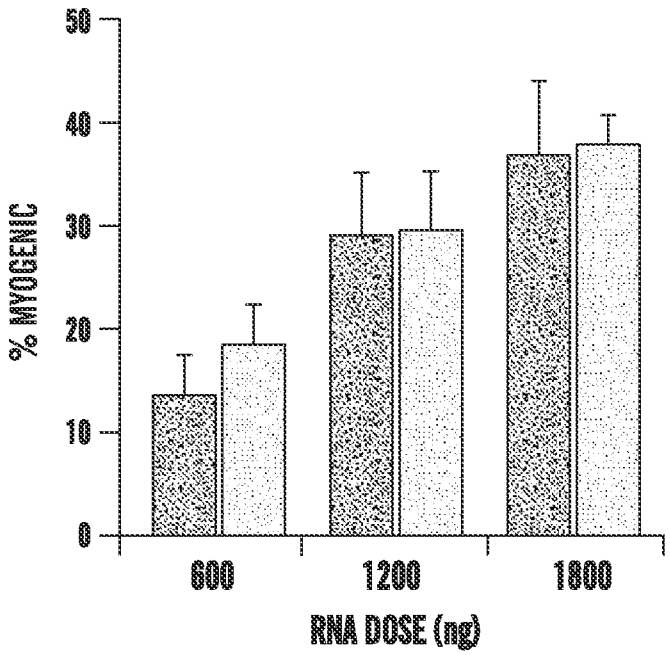
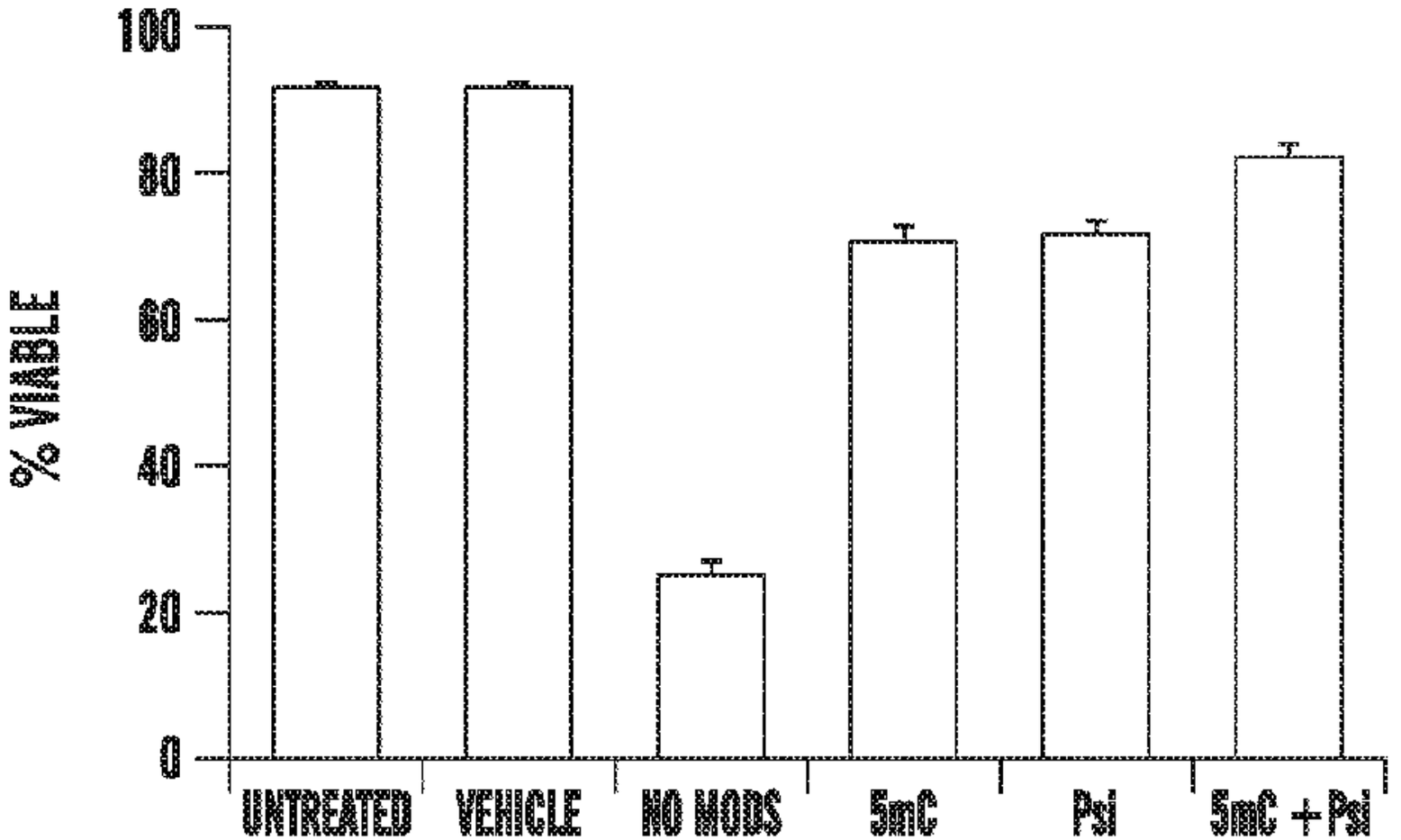


FIG. 10B


% VIABLE
100
80
60
40
20
0
UNTREATED
VEHICLE
NO MODS
5mC
Psi
5mC + Psi