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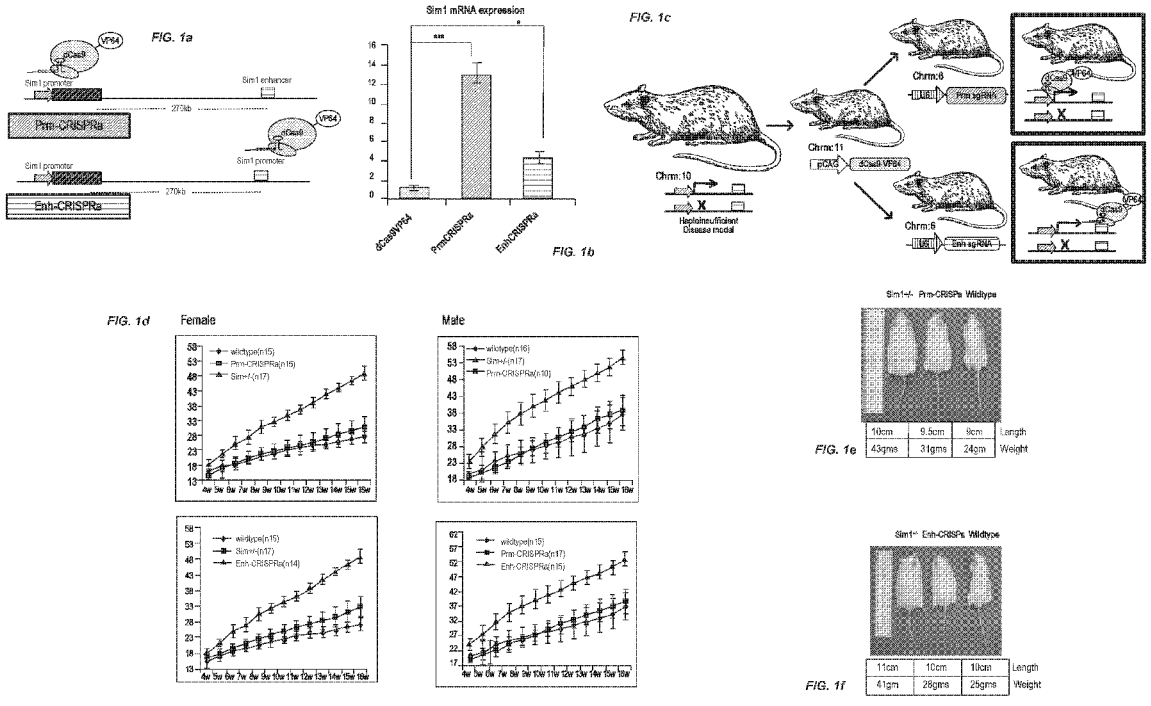

(57) Abstract: Methods and compositions are provided for activating transcription in a mammalian cell.

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GENE THERAPY FOR HAPLOINSUFFICIENCY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority to U.S. Provisional Application No. 62/455,988 filed February 7, 2017, the content of which is hereby incorporated by reference in its entirety for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with government support under grant No. R01 DK090382 awarded by The National Institutes of Health. The government has certain rights in the invention.

REFERENCE TO SUBMISSION OF A SEQUENCE LISTING

[0003] This application includes a Sequence Listing as a text file named "081906-224410PC-1072775_SequenceListing.txt" created February 6, 2018 and containing 107 kilobytes. The material contained in this text file is incorporated by reference in its entirety for all purposes.

FIELD OF INVENTION

[0004] The present disclosure relates generally to methods and compositions for activating transcription in mammalian cells.

BACKGROUND OF THE INVENTION

[0005] Genomic alterations resulting in reduced transcription or activity of one or more genes or gene products are a causative factor in a myriad of mammalian diseases. One such genomic alteration is haploinsufficiency, in which there is only one functional copy of a gene and that single copy does not produce enough of the gene product to produce a wild-type phenotype. Other diseases are caused by genomic alterations in one or both copies of a gene that alter the gene product so that it exhibits a reduction, but not elimination, in activity. In still other

diseases, genomic alterations reduce transcription or reduce transcript stability of one or both copies of a gene, such that there is insufficient gene product to produce a wild-type phenotype. Numerous approaches have been attempted to treat such diseases by augmenting the amount or activity of the one or more genes reduced in transcription or activity. Such approaches include delivery into the genome of a wild-type copy of the one or more genes. Recently, targeted introduction into a genome has been demonstrated using methods and compositions based on clustered regularly interspaced short palindromic repeats (CRISPR), Zinc Finger Nucleases (ZFNs) (see, Urnov *et al., Nat. Rev. Genet.*, 11:636-646 (2010) or transcription activator-like effector nucleases (TALENs) (see, Joung and Sander, *Nat. Rev. Mol. Cell Biol.*, 1:49-55 (2013). Other approaches for increasing transcription of one or more target genes include the use of antisense oligomers that promote constitutive splicing (see, US 2016/0298121). However, there remains a need for alternative methods and compositions for increasing the transcription of target genes to treat diseases caused by their reduced transcription, amount, or activity.

BRIEF SUMMARY OF THE INVENTION

[0001] The present invention is directed to methods and compositions for increasing transcription of target genes in a mammalian (*e.g.*, human) subject. The inventors have discovered that such increased transcription can be achieved with a transcription-activating guide-RNA (gRNA) construct (*e.g.*, as part of a dCAS9/gRNA complex) targeted to a promoter or enhancer region of a gene. Moreover, the inventors have discovered that transcriptional activation in amounts and for periods of time that are sufficient to treat a disease can be achieved with a non-integrating vector. In some cases, the methods and compositions for transcriptional activation do not covalently modify the genome of the host mammal by endonuclease cleavage, nicking, and/or repair. In some cases, the non-integrating vector is an episomal vector, such as an adeno associated viral vector.

[0006a] In one aspect, the present invention provides a method of treating a haploinsufficiency disease in a mammalian subject, the method comprising contacting a cell of the subject with a composition comprising: i) a catalytically inactive CRISPR nuclease fused to a transcriptional activation domain, and ii) a guide RNA, wherein the guide RNA comprises: a) a targeting region that, under conditions present in a nucleus of the cell, specifically hybridizes to a promoter

region or an enhancer region operably linked to a wild-type copy of a haploinsufficient gene; and b) a binding region that specifically binds the catalytically inactive CRISPR nuclease under conditions present in a nucleus of the cell; and wherein the contacting forms a complex comprising the catalytically inactive CRISPR nuclease bound to the guide RNA, wherein the targeting region of the guide RNA in the complex is hybridized to the promoter or enhancer of the wild-type copy of the haploinsufficient gene, and wherein the complex activates transcription of the wild-type copy of the haploinsufficient gene in an amount and for a duration sufficient to treat the haploinsufficiency disease in the subject.

[0002] In one aspect, the present invention provides a method of treating a haploinsufficiency disease in a mammalian subject, the method comprising contacting a cell of the subject with a composition comprising: i) a guide RNA, wherein the guide RNA comprises: a) a targeting region that, under conditions present in a nucleus of the cell, specifically hybridizes to a promoter region or an enhancer region operably linked to a wild-type copy of a haploinsufficient

gene; and b) a CRISPR nuclease-binding region that specifically binds a CRISPR nuclease under conditions present in a nucleus of the cell or a region that specifically binds to the CRISPR nuclease-binding region; and ii) the CRISPR nuclease, -wherein the contacting forms a complex comprising the CRISPR nuclease bound to the guide RNA, wherein the targeting region of the guide RNA in the complex is hybridized to the promoter or enhancer; -wherein the complex comprises a catalytically inactive CRISPR nuclease and a transcriptional activation domain, and -wherein the complex activates transcription of the wild-type copy of the haploinsufficient gene in an amount and for a duration sufficient to treat the haploinsufficiency disease in the subject. In some embodiments, the mammalian subject is treated with a host cell obtained from the subject. In one embodiment, the mammalian subject is treated with a host cell obtained from a different (distinct) mammalian subject. In some embodiments, the host cell is an isolated mammalian host cell. In another embodiment, the host cell comprises an isolated mammalian host cell having one functional copy of a target gene.

[0008] In some embodiments, the contacting comprises contacting the cell with an episomal vector encoding the guide RNA or the CRISPR nuclease. In some embodiments, the contacting comprises contacting the cell with an episomal vector encoding the guide RNA and the CRISPR nuclease. In some embodiments, the contacting comprises contacting the cell with an episomal vector encoding the guide RNA and a second episomal vector encoding the CRISPR nuclease. In some embodiments, the episomal vector(s) are non-integrating. In some embodiments, the episomal vector(s) are non-replicating. In some embodiments, the episomal vector(s) are adeno-associated virus (AAV) vectors. In some embodiments, the episomal vector(s) independently comprise a first and a second end, wherein the first end and second end each independently comprise an AAV inverted terminal repeat.

[0009] In some embodiments, the CRISPR nuclease comprises (i) a nuclease domain that has been modified to eliminate nuclease and nicking activity and (ii) a transcriptional activation domain. In some embodiments, the CRISPR nuclease comprises a Cas9 or Cpf1 nuclease. In some embodiments, the modification comprises a mutation at positions corresponding to D10 and H840 of *S. pyogenes* Cas9. In some embodiments, the CRISPR nuclease comprises a D10A, H840A *S. pyogenes* dCas9. In some embodiments, the CRISPR nuclease comprises a *S. aureus* dCas9. In some embodiments the *S. aureus* dCas9 comprises one or more mutations in one of

the following residues: E782, K929, N968, R1015. In some embodiments, the guide RNA comprises a dead guide sequence.

[0010] In some embodiments, the guide RNA comprises a transcriptional activation binding domain, wherein the transcriptional activation binding domain specifically binds a composition comprising one or more transcriptional activation domains. In some embodiments, the complex comprising the CRISPR nuclease bound to the guide RNA further comprises a transcriptional activation domain selected from the group consisting of HSF1, VP16, VP64, p65, MyoD1, RTA, SET7/9, VPR, histone acetyltransferase p300, an hydroxylase catalytic domain of a TET family protein (*e.g.*, TET1 hydroxylase catalytic domain), LSD1, CIB1, AD2, CR3, EKLF1, GATA4, PRVIE, p53, SP1, MEF2C, TAX, and PPARγ. In some embodiments, the CRISPR nuclease is a CRISPR nuclease-VP64 fusion polypeptide.

[0011] In some embodiments, the guide RNA comprises a scaffold region. In some embodiments, the scaffold region comprises an ms2, f6, PP7, com, or L7a ligand sequence. In some embodiments, the scaffold region of the guide RNA in the complex is bound to a transcriptional activation domain fused to an MCP polypeptide, a COM polypeptide, a PCP polypeptide, or an L7a polypeptide. In some embodiments, the haploinsufficient gene is *SIM1, Leptin, Leptin receptor, MC4R, SCN2A, SETD5, PAX6, PKD1, MC3R, POMC, STAT3, STAT5, SOCS3, GHR, NPY, NPY1R, NPY2R, NPY5R, PYY, AMPK (PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1, PRKAG2, PRKAG3), OXT, JAK2, SHP2, NOS3, NROB2, BRS3, CARTPT, FABP4, HTR2C, IL6, NHLH2, NMU, NPB, NPBWRI, PNPLA2, UCP3, ADIPOQ, APOA5, ARNT2, ASIP, C1QTNF2, C3AR1, CCK, CPT1B, CSF2, DGAT1, DGAT2, GHRL, GHSR, HSD11B1, HTR7, INSIG1, INSIG2, LIPC, NMURI, NMUR2, NPBWR2, NTS, PPARGC1A, PPY, RETN, SIRT1, TGFBR2, WDTC1, or FOXO1.*

[0012] In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:1 (GACACGGAATTCATTGCCAG), SEQ ID NO:2 (CTGCGGGTTAGGTCTACCGG), SEQ ID NO:3 (GTTGAGCGCTCAGTCCAGCG), SEQ ID NO:4 (TCCCGACGTCGTGCGCGACC), or SEQ ID NO:5 (GCTCTGAATCTTACTACCCG). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:6 (GCTGTTAACTAAAGACAGGG), SEQ ID NO:7 (GTGGTCTGGGTGATCTCATG), SEQ ID NO:8 (GACAAAGGAACATCTGAGAGG), SEQ

ID NO:9 (GTGATCTCATGGGGAAGAGG), or SEQ ID NO:10 (GGCTTTGATCGTGGTCTGGG). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:11 (GCGAGCCCAGTCGCGTGGGG), or SEQ ID NO:12 (GCCAAGAATTGGCCAAAGGG), SEQ ID NO:34 (GTCAAAGGGGCATATGGAAGG), SEQ ID NO:35 (GGGAAGAAAGCCCCACTTGG), SEQ ID NO:36 (GCCCAGTCGCGTGGGGGGGG), or SEQ ID NO:37 (GGAGCGCGAGTGTCACTCGG). In another embodiment, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:38 (GCTCACTGTAGGACCCGAGCC), SEQ ID NO:39 (GACGCGGCGCTCATTGGCCAA), SEQ ID NO:40 (CGAGCCGCGAGCCCAGTCGCG), SEQ ID NO:41 (TCCCCCCCCCCCCCCACGCGA), SEQ ID NO:42 (GTCACTCACCCCGATTGGCCA), or SEQ ID NO:43 (CGCGAGCCCAGTCGCGTGGGG). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:44 (GTTGGCTTATCCAAACATCTC), SEQ ID NO:45 (ATGTTAAGCAAGGGTAATAGA), SEQ ID NO:46 (CTGTGAAAGGAATACAATTCA), SEQ ID NO: 47 (GCCAATTCTTGGCAACCGAGC), SEQ ID NO:48 (GAATTGGCCAAAGGGAGGGGT), or SEQ ID NO:49 (AATTAGCAGACAGCTTGGTAC). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:50 (CTGGCTGATTCCCGAGGATTT), SEQ ID NO:51 (CACTGAATACGGATTGGTCAG), SEQ ID NO:52 (GATGTCTCAGAACCACTGAAT), SEQ ID NO:53 (AACCACTGAATACGGATTGGT), or SEQ ID NO:54 (ACCAATCCGTATTCAGTGGTT). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:55 (GGCGCGGGGCGGACGGGGCGA), SEQ ID NO:56 (GCGCCCCGGGAACGCGTGGGG), SEQ ID NO:57 (CGCCCCGCGCCGCGCGGGGAG), SEQ ID NO:58 (TCCGCCCCGCGCCGCGCGGGG), SEQ ID NO:59 (GGAACGCGTGGGGCGGAGCTT), SEQ ID NO:60 (GCCCCGCGCCGCGCGGGGAGG), SEQ ID NO:61 (TGCGCCCCGGGAACGCGTGGG), SEQ ID NO:62 (GAACGCGTGGGGCGGAGCTTC), SEQ ID NO:63 (GCGGCGCGGGGCGGACGGGGC), or SEQ ID NO:64 (CCCGTCCGCCCCGCGCCGCGC). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:65 (GGCCCACTCGCCGCCAATCAG), SEQ ID NO:66 (GGAAGCCGCCGGGGCCGCCTA),

SEQ ID NO:67 (TGATTGGCGGCGAGTGGGCCA), SEQ ID NO:68: (GCCGCCAATCAGCGGAAGCCG), SEQ ID NO:69: (GGCGGCTTCCGCTGATTGGCG), SEQ ID NO:70: (CCGCCAATCAGCGGAAGCCGC), SEQ ID NO:71: (AGCCGCCGGGGCCGCCTAGAG), SEQ ID NO:72: (GCTTCCGCTGATTGGCGGCGA), SEQ ID NO:73: (CGGCGAGTGGGCCAATGGGTG), or SEQ ID NO:74: (CCAATGGGTGCGGGGCGGTGG). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:75 (GGCTGCCGGGGCCGCCTAAAG), SEQ ID NO:76 (GGAGGCTGCCGGGGCCGCCTA), SEQ ID NO:77 (GCCGCCAATCAGCGGAGGCTG), SEQ ID NO:78 (CCGCCAATCAGCGGAGGCTGC), SEQ ID NO:79 (TGGCCGGTGCGCCGCCAATCA), SEQ ID NO:80 (GGCCGGTGCGCCGCCAATCAG), SEQ ID NO:81(CGGCGCACCGGCCAATAAGTG), SEQ ID NO:82(ATAAGTGTGGGGCGGTGGGCG), SEQ ID NO:83 (CCAATAAGTGTGGGGCGGTGG), or SEQ ID NO:84 (CAATAAGTGTGGGGCGGTGGG). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:85 (CCTTTCTATGACCTAGTCGG), SEQ ID NO:86 (CAGAATCAGTAACGCACTGT), SEQ ID NO:87 (GAAACCAGGAGAGATAACCC), SEQ ID NO:88 (GGACCCCAGATATTCTGGAA), SEQ ID NO:89 (TTATTGTTGACTTAACGAAG), SEQ ID NO:90 (AAAAAGAAGCAAATAGCTAA), or SEQ ID NO:91 (AGAATCAGTAACGCACTGTA). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:92 (TGTTGGTTTATTGGACCCCAGATATTC), SEQ ID NO:93 (TGTTGGAGAAAATTAACTTAGTGCATA), or SEQ ID NO:94 (TGTTGGTATAACTGCCACTAGAGGGCT). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to SEQ ID NO:95 (AGGAGCCGGGACCCACCGG).

[0013] In some embodiments, the cell is a non-dividing cell. In some embodiments, the cell is a neuron. In some embodiments, the cell is a hypothalamus cell. In some embodiments, the contacting comprises injection of nucleic acid encoding the guide RNA and/or the CRISPR nuclease into a region of a brain containing a hypothalamus. In some embodiments, the contacting comprises injection of an adeno-associated viral vector comprising nucleic acid

encoding the guide RNA and/or the CRISPR nuclease into a region of a brain containing a hypothalamus. In some embodiments, the haploinsufficiency disease is selected from Table 1. In some embodiments, the haploinsufficiency disease is selected from obesity, autism, epilepsy, intellectual disability, aniridia, and polycystic kidney disease. In some embodiments, the haploinsufficiency disease is obesity.

[0014] In another aspect, the present invention provides a mammalian host cell comprising: I.) a genome comprising at least one functional copy of a target gene, wherein the functional cop(y/ies) in the absence of transcriptional activation by a heterologous complex do not produce enough of a corresponding gene product to produce a wild-type phenotype in an organism; and II.) the heterologous complex, wherein the heterologous complex comprises: a) a guide RNA, wherein the guide RNA comprises: i.) a targeting region that specifically hybridizes to a promoter region or an enhancer region operably linked to the functional cop(y/ies) of the target gene under conditions present in a nucleus of the cell; and ii.) a CRISPR nuclease-binding region that specifically binds a CRISPR nuclease under conditions present in a nucleus of the cell; and b) the CRISPR nuclease, -wherein the guide RNA of the heterologous complex comprising the CRISPR nuclease bound to the guide RNA is hybridized to the promoter or enhancer; -wherein the CRISPR nuclease is catalytically inactive, and -wherein the complex activates transcription of the functional cop(y/ies) of the target gene in an amount and for a duration sufficient to produce a wild-type phenotype when the host cell is present in an organism.

[0015] In some embodiments, the genome comprises a single functional copy of the target gene. In some embodiments, the single functional copy of the target gene comprises a haploinsufficient gene. In some embodiments, the genome comprises less than two functional copies of the target gene.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **Figs. 1A-F: Transgenic CRISPRa *Sim1* overexpression *in vitro* and *in vivo*. A**, Schema of the mouse *Sim1* genomic locus. **B**, CRISPRa in Neuro-2A cells targeting the *Sim1* promoter (Pr) or enhancer (Enh). Results are expressed as mRNA fold-increase normalized to beta-actin using the ΔΔCT method. The mean values±s.d. were obtained from 3 independent experiments. * = p-value < 0.001 *** = p-value < 0.0005 (ANOVA, Tukey test). **C**, Schema

showing the various mouse lines and mouse transgenic CRISPRa concept. **D**, Weekly weight measurements of wild-type littermates, *Sim1*$^{+/-}$, *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{Sim1Pr\text{-}sgRNA}$ and *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{SCE2En\text{-}sgRNA}$. At least 10 male and female mice were measured per genotype. Mean values±s.d are shown. **E-F**, Pictures showing 20 week old mice for each genotype: *Sim1*$^{+/-}$, *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{Sim1Pr\text{-}sgRNA}$ and wild-type littermate (**E**) and *Sim1*$^{+/-}$, *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{SCE2En\text{-}sgRNA}$ and wild-type littermate (**F**). Length and weight of each mice are depicted above and below respectively.

[0017] Figs. 2A-D Body composition and metabolic analyses of *Sim1* CRISPRa transgenic mice. A, Estimated percent fat in wild-type littermates, *Sim1*$^{+/-}$, *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{Sim1Pr\text{-}sgRNA}$ (PrmCRISPRa) and *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{SCE2En\text{-}sgRNA}$ (EnhCRISPRa) as determined by Dual Energy X-ray Absorptiometry (DEXA) or Echo Magnetic Resonance Imaging (EchoMRI), with their corresponding body weight measurements. The mean values±s.d. were obtained from 3 females and 3 males. **B**, Metabolic chamber energy expenditure analyses for 3 males and 3 females for all four genotypes determined over a 4 day period. **C**, Food intake for all four genotypes determined over a 4 day period. Mean values ± s.d. were obtained from 3 females and 3 males. * = p-value < 0.001; *** = p-value < 0.0005; n.s = non-significant (ANOVA, Tukey test). **D**, Respiratory exchange ratio (RER; VCO2/VO2) for all four genotypes obtained from 3 females and 3 males and plotted as mean values ± s.d.

[0018] Figs. 3A-D *dCas9* and *Sim1* mRNA expression levels in CRISPRa transgenic mice. A, Heatmap of *Sim1* tissue expression. Red and grey filled squares signify tissues where *Sim1* is expressed and not expressed, respectively as determined in our wild-type mice. **B**, *dCas9* mRNA expression in the hypothalamus, kidney, lung and liver from 4 *Sim1*$^{+/-}$ X *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ mice. The mean values±s.d were determined based on mRNA fold-increase normalized to beta-actin (for hypothalamus) and Rpl38 (for kidney, lung, liver) using the ΔΔCT method. **C-D**, Sim1 mRNA expression in the hypothalamus, kidney, lung and liver for the following genotypes: wild-type littermates, *Sim1*$^{+/-}$, *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{Sim1Pr\text{-}sgRNA}$ (Prm-CRISPRa) and *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{SCE2En\text{-}sgRNA}$ (Enh-CRISPRa) from 2 females (**C**) and 2 male (**D**). The mean values±s.d were determined based on mRNA fold-increase compared to wild-type littermates and normalized to beta-actin or Rpl38 using the ΔΔCT method. B.D.L = below detected levels.

[0019] Figs. 4A-E CRISPRa *Sim1* overexpression *in vitro* and *in vivo* using AAV. A, AAV CRISPRa in Neuro-2A cells using virons containing: *pCMV-dCas9-VP64* (dCas9-VP64), *pCMV-dCas9-VP64* along with *pSim1Pr-mCherry* (PrmCRIPSRa) and *pCMV-dCas9-VP64* along with *pSCE2En-mCherry* (EnhCRISPRa). Results are expressed as mRNA fold-increase normalized to beta-actin using the ΔΔCT method. The mean values±s.d. were obtained from 3 independent experiments. *** = p-value < 0.0005 (ANOVA, Tukey test). **B**, Schema showing the PVN injected region. **C**, Immunohistochemistry of *pSim1Pr-mCherry* injected hypothalamus from 20 week old mice showing mCherry expression in the PVN. **D-E**, *Cas9* (d) and *Sim1* (e) mRNA expression from *pCMV-dCas9-VP64* (dCas9-VP64), *pCMV-dCas9-VP64* + *pSim1Pr-mCherry* (PrmCRIPSRa, n=3) and *pCMV-dCas9-VP64* + *pSCE2En-mCherry* (EnhCRISPRa, n=4) from injected mice. The mean values±s.d were determined based on mRNA fold-increase compared to $Sim1^{+/-}$ mice and normalized to beta-actin using the ΔΔCT method.

[0020] Figs. 5A-C CRISPRa-AAV injection in PVN reduces weight gain in *Sim1*+/- mice. A, Timeline for weight measurement post CRISPRa-AAV injection in PVN. **B-C**, Weight gain determined over a 7 week period from $Sim1^{+/-}$ mice injected with *pCMV-dCas9-VP64* (dCas9-VP64), *pCMV-dCas9-VP64* + *pSim1Pr-mCherry* (Prm-CRIPSRa) *pCMV-dCas9-VP64* + *pSCE2En-mCherry* (Enh-CRISPRa) compared to un-injected wild-type littermates and $Sim1^{+/-}$ mice. Mean values±s.d are shown from 3 females (**B**) and 3 males (**C**). * = p-value < 0.001 *** = p-value < 0.0005 n.s = non-significant; (ANOVA, Tukey test).

[0021] Fig. 6 Schema of CRISPRa haploinsufficiency rescue experiments. The obesity phenotype in $Sim1^{+/-}$ mice was rescued via CRISPRa by targeting either the *Sim1* promoter or enhancer using both a transgenic and postnatal AAV approach**.**

[0022] Fig. 7A-7B: CRISPRa *Sim1* overexpression *in vitro*. Fig. 7A, shows an exemplary *S. aureus* CRISPRa system targeting the *Sim1* promoter (Pr) by transfection of various sgRNA's (SEQ ID NOS:38-43) into Neuro-2A (N2A) cells. Results are expressed as mRNA fold-increase normalized to Sa-dCas9-VP64. The mean values±s.d. were obtained from 3 independent experiments. **Fig. 7B**, shows an exemplary *S. aureus* CRISPRa in N2A cells targeting the *Sim1* promoter (Pr) after infection of AAV's containing select sgRNA's (SEQ ID NOS:38, 40, or 42) into N2A cells. Results are expressed as mRNA fold-increase normalized to VP64 alone. The mean values±s.d. were obtained from 3 independent experiments.

[0023] Fig. 8A-8B: CRISPRa *Sim1* overexpression *in vitro*. **Fig. 8A**, shows an exemplary *S. aureus* CRISPRa system targeting the *Sim1* SCE2 enhancer (Enh) by transfection of various sgRNA's (SEQ ID NOS:44-49) into N2A cells. Results are expressed as mRNA fold-increase normalized to Sa-dCas9-VP64. The mean values±s.d. were obtained from 3 independent experiments. **Fig. 8B**, shows an exemplary *S. aureus* CRISPRa system targeting the *Sim1* SCE2 enhancer (Enh) after infection of AAV's containing select sgRNA's (SEQ ID NOS:45, 46, or 47) into N2A cells. Results are expressed as mRNA fold-increase normalized to VP64 alone. The mean values±s.d. were obtained from 3 independent experiments.

[0024] Fig. 9A-9B: CRISPRa *Mc4r* overexpression *in vitro*. **Fig. 9A**, shows an exemplary *S. aureus* CRISPRa system targeting the *Mc4r* promoter (Pr) by transfection of various sgRNA's (SEQ ID NOS:50-54) into N2A cells. Results are expressed as mRNA fold-increase normalized to VP64. The mean values±s.d. were obtained from 3 independent experiments. **Fig. 9B**, shows an exemplary *S. aureus* CRISPRa system targeting the *Mc4r* promoter (Pr) after infection of AAV's containing select sgRNA's (SEQ ID NOS:51, 52, or 54) into N2A cells. Results are expressed as mRNA fold-increase normalized to VP64. The mean values±s.d. were obtained from 3 independent experiments.

[0025] Fig. 10: CRISPRa *PKD1* overexpression *in vitro*. An exemplary *S. aureus* CRISPRa system targeting the *PKD1* promoter (Pr) by transfection of human promoter sgRNA's (SEQ ID NOS:55-64) into human HEK293T cells. Results are expressed as mRNA fold-increase normalized to dCas9-VP64. The mean values±s.d. were obtained from 3 independent experiments.

[0026] Fig. 11A-11B: CRISPRa *SETD5* overexpression *in vitro*. **Fig. 11A,** shows an exemplary *S. aureus* CRISPRa system targeting the *SETD5* promoter (Pr) or THUMPD3 by transfection of human promoter sgRNA's (SEQ ID NOS:65-74) into human HEK293T cells. HS MIX refers to transfection of an equimolar concentration of each of HS01-HS10 into human HEK293T cells. Results are expressed as mRNA fold-increase normalized to VP64 alone. The mean values±s.d. were obtained from 3 independent experiments. **Fig. 11B,** shows an exemplary *S. aureus* CRISPRa system targeting the *SETD5* promoter (Pr) or ROSA26 by transfection of mouse promoter sgRNA's (SEQ ID NOS:75-84) into mouse Neuro-2A cells. MS MIX refers to transfection of an equimolar concentration of each of MS01-MS10 into mouse Neuro-2A cells.

Results are expressed as mRNA fold-increase normalized to VP64 alone. The mean values±s.d. were obtained from 3 independent experiments.

[0027] Fig. 12A-12B: CRISPRa *Scn2A* overexpression *in vitro*. Fig. 12A, shows an exemplary *S. pyogenes* (Sp) Cas9 CRISPRa system targeting the *Scn2a* promoter (Pr) by transfection of various sgRNA's (SEQ ID NOS:85-91) into N2A cells. Results are expressed as mRNA fold-increase normalized to VP64 alone. The mean values±s.d. were obtained from 3 independent experiments. **Fig. 12B**, shows an exemplary *S. aureus* CRISPRa system targeting the *Scn2a* promoter (Pr) after infection of AAV's containing select sgRNA's (SEQ ID NOS:92-94) into N2A cells. Two different multiplicity of infection (MOI) were used: 5,000 and 1,250 viral genome (vg/ml). Results are expressed as mRNA fold-increase normalized to VP64 alone. The mean values±s.d. were obtained from 3 independent experiments.

[0028] Fig. 13: CRISPRa *PAX6* overexpression *in vitro*. shows an exemplary *S. pyogenes* (Sp) Cas9 CRISPRa system targeting the *PAX6* promoter (Pr) by lentiviral delivery of human promoter sgRNA (SEQ ID NO:95) into human H1-ESC cells differentiated into neurons. Results are expressed as relative expression to HPRT. The mean values±s.d. were obtained from 3 independent experiments. Additional neuronal markers are shown to demonstrate that *PAX6* CRISPRa leads to neural induction of H1-ESCs.

DEFINITIONS

[0029] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

[0030] "Treating" refers to any indicia of success in the treatment or amelioration or prevention of the disease, condition, or disorder, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the disease condition more tolerable to the patient; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of an examination by a physician. Accordingly, the term "treating" includes the administration of the compounds or agents of the present invention to prevent or delay, to alleviate, or to arrest or inhibit development of the symptoms or conditions associated with a disease, condition or disorder as described herein. The

term “therapeutic effect” refers to the reduction, elimination, or prevention of the disease, symptoms of the disease, or side effects of the disease in the subject. “Treating” or “treatment” using the methods of the present invention includes preventing the onset of symptoms in a subject that can be at increased risk of a disease or disorder associated with a disease, condition or disorder as described herein, but does not yet experience or exhibit symptoms, inhibiting the symptoms of a disease or disorder (slowing or arresting its development), providing relief from the symptoms or side-effects of a disease (including palliative treatment), and relieving the symptoms of a disease (causing regression). Treatment can be prophylactic (to prevent or delay the onset of the disease, or to prevent the manifestation of clinical or subclinical symptoms thereof) or therapeutic suppression or alleviation of symptoms after the manifestation of the disease or condition. The term “treatment,” as used herein, includes preventative (e.g., prophylactic), curative or palliative treatment.

[0031] The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologues, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al, Mol. Cell. Probes 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

[0032] The term "gene" means the segment of DNA involved in producing a polypeptide chain. It may include regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

[0033] A "promoter" is defined as an array of nucleic acid control sequences that direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid

sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription.

[0034] An "expression cassette" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular polynucleotide sequence in a host cell. An expression cassette may be part of a plasmid, viral genome, or nucleic acid fragment. Typically, an expression cassette includes a polynucleotide to be transcribed, operably linked to a promoter.

[0035] A "reporter gene" encodes proteins that are readily detectable due to their biochemical characteristics, such as enzymatic activity or chemifluorescent features. One specific example of such a reporter is green fluorescent protein. Fluorescence generated from this protein can be detected with various commercially-available fluorescent detection systems. Other reporters can be detected by staining. The reporter can also be an enzyme that generates a detectable signal when contacted with an appropriate substrate. The reporter can be an enzyme that catalyzes the formation of a detectable product. Suitable enzymes include, but are not limited to, proteases, nucleases, lipases, phosphatases and hydrolases. The reporter can encode an enzyme whose substrates are substantially impermeable to eukaryotic plasma membranes, thus making it possible to tightly control signal formation. Specific examples of suitable reporter genes that encode enzymes include, but are not limited to, CAT (chloramphenicol acetyl transferase; Alton and Vapnek (1979) Nature 282: 864-869); luciferase (lux); β-galactosidase; LacZ; β. -glucuronidase; and alkaline phosphatase (Toh, et al. (1980) Eur. J. Biochem. 182: 231-238; and Hall et al. (1983) J. Mol. Appl. Gen. 2: 101), each of which are incorporated by reference herein in its entirety. Other suitable reporters include those that encode for a particular epitope that can be detected with a labeled antibody that specifically recognizes the epitope.

[0036] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g. , hydroxyproline, γ- carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic

chemical structure as a naturally occurring amino acid, i.e., an a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups {e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. "Amino acid mimetics" refers to chemical compounds having a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0037] There are various known methods in the art that permit the incorporation of an unnatural amino acid derivative or analog into a polypeptide chain in a site-specific manner, see, e.g., WO 02/086075.

[0038] Amino acids may be referred to herein by either the commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0039] "Polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. All three terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non- naturally occurring amino acid polymers. As used herein, the terms encompass amino acid chains of any length, including full-length proteins, wherein the amino acid residues are linked by covalent peptide bonds.

[0040] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, "conservatively modified variants" refers to those nucleic acids that encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one

species of conservatively modified variations. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[0041] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention. In some cases, conservatively modified variants of a CRISPR nuclease such as Cas9 or a guide RNA such as a small guide RNA (sgRNA) can have an increased stability, assembly, or activity as described in WO 2016/011080, the contents of which are hereby incorporated by reference in the entirety for all purposes including, without limitation, the sgRNAs, sgRNA scaffolds, sgRNA libraries, and sgRNA binding regions described therein.

[0042] The following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);

7) Serine (S), Threonine (T); and

8) Cysteine (C), Methionine (M)

(see, e.g., Creighton, Proteins, W. H. Freeman and Co., N. Y. (1984)).

[0043] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0044] In the present application, amino acid residues are numbered according to their relative positions from the left most residue, which is numbered 1 , in an unmodified wild- type polypeptide sequence.

[0045] As used in herein, the terms "identical" or percent "identity," in the context of describing two or more polynucleotide or amino acid sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same. For example, a core small guide RNA (sgRNA) sequence responsible for assembly and activity of a sgRNA :nuclease complex has at least 80% identity, preferably 85%, 90%, 91%, 92%, 93, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identity, to a reference sequence, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection.

[0046] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. For sequence comparison of nucleic acids and proteins, the BLAST and BLAST 2.0 algorithms and the default parameters discussed below are used.

[0047] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually

about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., Current Protocols in Molecular Biology (Ausubel et al., eds. 1995 supplement)).

[0048] Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al, (1990) J. Mol. Biol. 215: 403-410 and Altschul et al. (1977) Nucleic Acids Res. 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available at the National Center for Biotechnology Information website, ncbi.nlm.nih.gov. The algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive- valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al, supra). These initial neighborhood word hits acts as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word size (W) of 28, an expectation (E) of 10, M=l, N=-

2, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word size (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89: 10915 (1989)).

[0049] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences {see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0050] An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence. Yet another indication that two polypeptides are substantially identical is that the two polypeptides retain identical or substantially similar activity.

[0051] A "translocation sequence" or "transduction sequence" refers to a peptide or protein (or active fragment or domain thereof) sequence that directs the movement of a protein from one cellular compartment to another, or from the extracellular space through the cell or plasma membrane into the cell. Translocation sequences that direct the movement of a protein from the extracellular space through the cell or plasma membrane into the cell are "cell penetration peptides." Translocation sequences that localize to the nucleus of a cell are termed "nuclear localization" sequences, signals, domains, peptides, or the like.

[0052] Examples of translocation sequences include, without limitation, the TAT transduction domain (see, e.g., S. Schwarze et al, Science 285 (Sep. 3, 1999); penetratins or penetratin

peptides (D. Derossi et al, Trends in Cell Biol. 8, 84-87); Herpes simplex virus type 1 VP22 (A. Phelan et al., Nature Biotech. 16, 440-443 (1998), and polycationic (e.g., poly-arginine) peptides (Cell Mol. Life Sci. 62 (2005) 1839-1849). Further translocation sequences are known in the art. Translocation peptides can be fused (e.g. at the amino or carboxy terminus), conjugated, or coupled to a compound of the present invention, to, among other things, produce a conjugate compound that may easily pass into target cells, or through the blood brain barrier and into target cells.

[0053] As used herein, the term "CRISPR" refers to any one of the naturally occurring Clustered Regularly Interspaced Short Palindromic Repeat systems or loci, or a derivative thereof. CRISPR loci can be found in the genomes of many bacteria and archaea. There are four types of CRISPR systems (e.g., Type I, Type II, Type III, and Type U).

[0054] A CRISPR locus can comprise polynucleotide sequences encoding for CRISPR Associated Genes (Cas) genes. Cas genes can be involved in the biogenesis and/or the interference stages of crRNA function. Cas genes can be named according to the organism from which they are derived. For example, Cas genes in *Staphylococcus epidermidis* can be referred to as Csm-type, Cas genes in *Streptococcus thermophilus* can be referred to as Csn-type, and Cas genes in *Pyrococcus furiosus* can be referred to as Cmr-type.

[0055] As used herein, the term CRISPR nuclease refers to a polypeptide of, or derived from, a nuclease encoded in any one of the four types of CRISPR loci: Type I, Type II, Type III, and Type U, wherein the natural sequence of the polypeptide exhibits RNA-guided nuclease activity. A CRISPR nuclease can be catalytically inactive. Catalytically inactive CRISPR nucleases do not exhibit nuclease or nickase activity when in complex with an RNA-guide and bound to a nucleic acid target containing a target domain and, in certain embodiments, a PAM sequence. The catalytically inactive CRISPR nuclease can be catalytically inactive due to one or more mutations of the CRISPR nuclease polypeptide sequence, or due to forming a complex with a guide RNA that is sufficient to provide RNA-guided targeting, but insufficient to support catalytic activity (*i.e.*, nuclease or nicking activity). For example, the CRISPR nuclease can be a wild-type CRISPR nuclease (*e.g.*, a Cas9 or Cpf1 nuclease) in complex with a dead guide sequence. For example, Cpf1 is a Class II CRISPR-Cas system and is described in Zetsche *et al.*, *Cell*, 163:759-771 (2015). Dead guide sequences and their use are further described in, *e.g.*,

WO 2016/094872, which is hereby incorporated by reference for all purposes, including dead guide sequences, complexes between CRISPR nucleases and dead guide sequences, and methods and compositions for making and using such dead guide sequences and complexes containing them.

[0056] In certain embodiments, a CRISPR nuclease meets one or both of the following criteria: it has at least 20, 30, 40, 50, 55, 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% homology with, or it differs by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 35, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350 or 400, amino acid residues from, the amino acid sequence of a reference sequences, e.g., a naturally occurring CRISPR nuclease. Additional CRISPR nucleases include, without limitation, one or more CRISPR nucleases described in WO 2016/154579.

[0057] In certain embodiments, a CRISPR nuclease contains (*i.e.*, is covalently or non-covalently linked to) one or more additional polypeptides or nucleic acids. For example, the CRISPR nuclease can be fused at an amino or carboxy-terminus to one or more transcriptional activation domain polypeptides, one or more DNA-binding polypeptides, one or more affinity tags (*e.g.*, in complex with one or more affinity tag ligands, such as affinity tag ligand-transcriptional activation domain fusion protein(s)), nuclear localization sequences, or a combination thereof.

[0058] Exemplary DNA-binding polypeptides include, but are not limited to, the programmable DNA binding domains described in Bolukbasi *et al.*, Nature Methods 12, 1150–1156 (2015), the contents of which are hereby incorporated by reference in the entirety including, *e.g.*, the programmable DNA-binding domains (pDBD), Cas9 variants, and Cas9-pDBD chimeras described therein. Exemplary transcriptional activation domain polypeptides include, but are not limited to, an activation domain of, or combinations of activation domains of, one or more of the following:

- heat shock transcription factor 1 (HSF1), *e.g.*, SEQ ID NO:13 (EKCLSVACLDKNELSDHLDAMDSNLDNLQTMLSSHGFSVDTSALLDLFSPSVTV PDMSLPDLDSSLASIQELLSPQEPPRPPEAENSSPDSGKQLVHYTAQPLFLLDPGS VDTGSNDLPVLFELGEGSYFSEGDGFAEDPTISLLTGSEPPKAKDPTVS)

- viral protein 16 (VP16), *e.g.*, SEQ ID NO:14 (DALDDFDLDML);
- tetrameric VP16 (VP64), *e.g.*, SEQ ID NO:15 (DALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDML)
- the p65 NF-Kβ transactivating subunit (p65), *e.g.*, SEQ ID NO:16 (SQYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASV PKPAPQPYPFTSSLSTINYDEFPTMVFPSGQISQASALAPAPPQVLPQAPAPAPAPA MVSALAQAPAPVPVLAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQFDDEDLGA LLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVAPHTTEPMLMEYPEAITRLVTGA QRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALL)
- MyoD1, *e.g.*, SEQ ID NO:17 (MELLSPPLRDIDLTGPDGSLCSFETADDFYDDPCFDSPDLRFFEDLDPRLVHMGA LLKPEEHAHFPTAVHPGPGAREDEHVRAPSGHHQAGRCLLWACKACKRKTTNA DRRKAATMRERRRLSKVNEAFETLKRCTSSNPNQRLPKVEILRNAIRYIEGLQAL LRDQDAAPPGAAAFYAPGPLPPGRGSEHYSGDSDASSPRSNCSDGMMDYSGPPS GPRRQNGYDTAYYSEAARESRPGKSAAVSSLDCLSSIVERISTDSPAAPALLLAD APPESPPGPPEGASLSDTEQGTQTPSPDAAPQCPAGSNPNAIYQVL)
- RTA, *e.g.*, SEQ ID NO:18 (RDSREGMFLPKPEAGSAISDVFEGREVCQPKRIRPFHPPGSPWANRPLPASLAPTP TGPVHEPVGSLTPAPVPQPLDPAPAVTPEASHLLEDPDEETSQAVKALREMADTV IPQKEEAAICGQMDLSHPPPRGHLDELTTTLESMTEDLNLDSPLTPELNEILDTFLN DECLLHAMHISTGLSIFDTSLF)
- SET7, *e.g.*, SEQ ID NO:19 (MDSDDEMVEEAVEGHLDDDGLPHGFCTVTYSSTDRFEGNFVHGEKNGRGKFFF FDGSTLEGYYVDDALQGQGVYTYEDGGVLQGTYVDGELNGPAQEYDTDGRLIF KGQYKDNIRHGVCWIYYPDGGSLVGEVNEDGEMTGEKIAYVYPDERTALYGKFI DGEMIEGKLATLMSTEEGRPHFELMPGNSVYHFDKSTSSCISTNALLPDPYESER VYVAESLISSAGEGLFSKVAVGPNTVMSFYNGVRITHQEVDSRDWALNGNTLSL DEETVIDVPEPYNHVSKYCASLGHKANHSFTPNCIYDMFVHPRFGPIKCIRTLRA VEADEELTVAYGYDHSPPGKSGPEAPEWYQVELKAFQATQQK)

- VPR, *e.g.*, SEQ ID NO:20
(EASGSGRADALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDD
FDLDMLINSRSSGSPKKKRKVGSQYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFS
GPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFPSGQISQAS
ALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPKPTQA
GEGTLSEALLQLQFDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVAP
HTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADMD
FSALLGSGSGSRDSREGMFLPKPEAGSAISDVFEGREVCQPKRIRPFHPPGSPWAN
RPLPASLAPTPTGPVHEPVGSLTPAPVPQPLDPAPAVTPEASHLLEDPDEETSQAV
KALREMADTVIPQKEEAAICGQMDLSHPPPRGHLDELTTTLESMTEDLNLDSPLT
PELNEILDTFLNDECLLHAMHISTGLSIFDTSLF)

- histone acetyltransferase p300, *e.g.*, SEQ ID NO:21
(KFSAKRLPSTRLGTFLENRVNDFLRRQNHPESGEVTVRVVHASDKTVEVKPGM
KARFVDSGEMAESFPYRTKALFAFEEIDGVDLCFFGMHVQEYGSDCPPPNQRRV
YISYLDSVHFFRPKCLRTAVYHEILIGYLEYVKKLGYTTGHIWACPPSEGDDYIFH
CHPPDQKIPKPKRLQEWYKKMLDKAVSERIVHDYKDIFKQATEDRLTSAKELPY
FEGDFWPNVLEESIKELEQEEEERKREENTSNESTDVTKGDSKNAKKKNNKKTS
KNKSSLSRGNKKKPGMPNVSNDLSQKLYATMEKHKEVFFVIRLIAGPAANSLPPI
VDPDPLIPCDLMDGRDAFLTLARDKHLEFSSLRRAQWSTMCMLVELHTQSQ)

- an hydroxylase catalytic domain of a TET family protein (*e.g.*, TET1 hydroxylase catalytic domain), *e.g.*, SEQ ID NO:22
(MSRSRHARPSRLVRKEDVNKKKKNSQLRKTTKGANKNVASVKTLSPGKLKQLI
QERDVKKKTEPKPPVPVRSLLTRAGAARMNLDRTEVLFQNPESLTCNGFTMALR
STSLSRRLSQPPLVVAKSKKVPLSKGLEKQHDCDYKILPALGVKHSENDSVPMQ
DTQVLPDIETLIGVQNPSLLKGKSQETTQFWSQRVEDSKINIPTHSGPAAEILPGPL
EGTRCGEGLFSEETLNDTSGSPKMFAQDTVCAPFPQRATPKVTSQGNPSIQLEEL
GSRVESLKLSDSYLDPIKSEHDCYPTSSLNKVIPDLNLRNCLALGGSTSPTSVIKFL
LAGSKQATLGAKPDHQEAFEATANQQEVSDTTSFLGQAFGAIPHQWELPGADPV
HGEALGETPDLPEIPGAIPVQGEVFGTILDQQETLGMSGSVVPDLPVFLPVPPNPIA
TFNAPSKWPEPQSTVSYGLAVQGAIQILPLGSGHTPQSSSNSEKNSLPPVMAISNV

ENEKQVHISFLPANTQGFPLAPERGLFHASLGIAQLSQAGPSKSDRGSSQVSVTST
VHVVNTTVVTMPVPMVSTSSSSYTTLLPTLEKKKRKRCGVCEPCQQKTNCGECT
YCKNRKNSHQICKKRKCEELKKKPSVVVPLEVIKENKRPQREKKPKVLKADFDN
KPVNGPKSESMDYSRCGHGEEQKLELNPHTVENVTKNEDSMTGIEVEKWTQNK
KSQLTDHVKGDFSANVPEAEKSKNSEVDKKRTKSPKLFVQTVRNGIKHVHCLPA
ETNVSFKKFNIEEFGKTLENNSYKFLKDTANHKNAMSSVATDMSCDHLKGRSNV
LVFQQPGFNCSSIPHSSHSIINHHASIHNEGDQPKTPENIPSKEPKDGSPVQPSLLSL
MKDRRLTLEQVVAIEALTQLSEAPSENSSPSKSEKDEESEQRTASLLNSCKAILYT
VRKDLQDPNLQGEPPKLNHCPSLEKQSSCNTVVFNGQTTTLSNSHINSATNQAST
KSHEYSKVTNSLSLFIPKSNSSKIDTNKSIAQGIITLDNCSNDLHQLPPRNNEVEYC
NQLLDSSKKLDSDDLSCQDATHTQIEEDVATQLTQLASIIKINYIKPEDKKVESTP
TSLVTCNVQQKYNQEKGTIQQKPPSSVHNNHGSSLTKQKNPTQKKTKSTPSRDR
RKKKPTVVSYQENDRQKWEKLSYMYGTICDIWIASKFQNFGQFCPHDFPTVFGK
ISSSTKIWKPLAQTRSIMQPKTVFPPLTQIKLQRYPESAEEKVKVEPLDSLSLFHLK
TESNGKAFTDKAYNSQVQLTVNANQKAHPLTQPSSPPNQCANVMAGDDQIRFQ
QVVKEQLMHQRLPTLPGISHETPLPESALTLRNVNVVCSGGITVVSTKSEEEVCSS
SFGTSEFSTVDSAQKNFNDYAMNFFTNPTKNLVSITKDSELPTCSCLDRVIQKDK
GPYYTHLGAGPSVAAVREIMENRYGQKGNAIRIEIVVYTGKEGKSSHGCPIAKW
VLRRSSDEEKVLCLVRQRTGHHCPTAVMVVLIMVWDGIPLPMADRLYTELTENL
KSYNGHPTDRRCTLNENRTCTCQGIDPETCGASFSFGCSWSMYFNGCKFGRSPSP
RRFRIDPSSPLHEKNLEDNLQSLATRLAPIYKQYAPVAYQNQVEYENVARECRLG
SKEGRPFSGVTACLDFCAHPHRDIHNMNNGSTVVCTLTREDNRSLGVIPQDEQL
HVLPLYKLSDTDEFGSKEGMEAKIKSGAIEVLAPRRKKRTCFTQPVPRSGKKRAA
MMTEVLAHKIRAVEKKPIPRIKRKNNSTTTNNSKPSSLPTLGSNTETVQPEVKSET
EPHFILKSSDNTKTYSLMPSAPHPVKEASPGFSWSPKTASATPAPLKNDATASCGF
SERSSTPHCTMPSGRLSGANAAAADGPGISQLGEVAPLPTLSAPVMEPLINSEPST
GVTEPLTPHQPNHQPSFLTSPQDLASSPMEEDEQHSEADEPPSDEPLSDDPLSPAE
EKLPHIDEYWSDSEHIFLDANIGGVAIAPAHGSVLIECARRELHATTPVEHPNRNH
PTRLSLVFYQHKNLNKPQHGFELNKIKFEAKEAKNKKMKASEQKDQAANEGPE
QSSEVNELNQIPSHKALTLTHDNVVTVSPYALTHVAGPYNHWV)

- LSD1, *e.g.*, SEQ ID NO:23
(GMDVTLLEARDRVGGRVATFRKGNYVADLGAMVVTGLGGNPMAVVSKQVN
MELAKIKQKCPLYEANGQAVPKEKDEMVEQEFNRLLEATSYLSHQLDFNVLNN
KPVSLGQALEVVIQLQEKHVKDEQIEHWKKIVKTQEELKELLNKMVNLKEKIKE
LHQQYKEASEVKPPRDITAEFLVKSKHRDLTALCKEYDELAETQGKLEEKLQELE
ANPPSDVYLSSRDRQILDWHFANLEFANATPLSTLSLKHWDQDDDFEFTGSHLT
VRNGYSCVPVALAEGLDIKLNTAVRQVRYTASGCEVIAVNTRSTSQTFIYKCDA
VLCTLPLGVLKQQPPAVQFVPPLPEWKTSAVQRMGFGNLNKVVLCFDRVFWDP
SVNLFGHVGSTTASRGELFLFWNLYKAPILLALVAGEAAGIMENISDDVIVGRCL
AILKGIFGSSAVPQPKETVVSRWRADPWARGSYSYVAAGSSGNDYDLMAQPITP
GPSIPGAPQPIPRLFFAGEHTIRNYPATVHGALLSGLREAGRIADQFLGAMYTLPR
QATPGVPAQQSPSM)

- CIB1, *e.g.*, SEQ ID NO:24
(MGGSGSRLSKELLAEYQDLTFLTKQEILLAHRRFCELLPQEQRSVESSLRAQVPF
EQILSLPELKANPFKERICRVFSTSPAKDSLSFEDFLDLLSVFSDTATPDIKSHYAFR
IFDFDDDGTLNREDLSRLVNCLTGEGEDTRLSASEMKQLIDNILEESDIDRDGTIN
LSEFQHVISRSPDFASSFKIVL)

- AD2, *e.g.*, SEQ ID NO:25
(MNQPQRMAPVGTDKELSDLLDFSMMFPLPVTNGKGRPASLAGAQFGGSGLED
RPSSGSWGSGDQSSSSFDPSRTFSEGTHFTESHSSLSSSTFLGPGLGGKSGERGAY
ASFGRDAGVGGLTQAGFLSGELALNSPGPLSPSGMKGTSQYYPSYSGSSRRRAA
DGSLDTQPKKVRKVPPGLPSSVYPPSSGEDYGRDATAYPSAKTPSSTYPAPFYVA
DGSLHPSAELWSPPGQAGFGPMLGGGSSPLPLPPGSGPVGSSGSSSTFGGLHQHE
RMGYQLHGAEVNGGLPSASSFSSAPGATYGGVSSHTPPVSGADSLLGSRGTTAG
SSGDALGKALASIYSPDHSSNNFSSSPSTPVGSPQGLAGTSQWPRAGAPGALSPSY
DGGLHGLQSKIEDHLDEAIHVLRSHAVGTAGDMHTLLPGHGALASGFTGPMSLG
GRHAGLVGGSHPEDGLAGSTSLMHNHAALPSQPGTLPDLSRPPDSYSGLGRAGA
TAAASEIKREEKEDEENTSAADHSEEEKKELKAPRARTSPDEDEDDLLPPEQKAE
REKERRVANNARERLRVRDINEAFKELGRMCQLHLNSEKPQTKLLILHQAVSVIL

NLEQQVRERNLNPKAACLKRREEEKVSGVVGDPQMVLSAPHPGLSEAHNPAGH
M)

- CR3, *e.g.*, SEQ ID NO:26
(MGPTSGPSLLLLLLTHLPLALGSPMYSIITPNILRLESEETMVLEAHDAQGDVPVT
VTVHDFPGKKLVLSSEKTVLTPATNHMGNVTFTIPANREFKSEKGRNKFVTVQA
TFGTQVVEKVVLVSLQSGYLFIQTDKTIYTPGSTVLYRIFTVNHKLLPVGRTVMV
NIENPEGIPVKQDSLSSQNQLGVLPLSWDIPELVNMGQWKIRAYYENSPQQVFST
EFEVKEYVLPSFEVIVEPTEKFYYIYNEKGLEVTITARFLYGKKVEGTAFVIFGIQD
GEQRISLPESLKRIPIEDGSGEVVLSRKVLLDGVQNPRAEDLVGKSLYVSATVILH
SGSDMVQAERSGIPIVTSPYQIHFTKTPKYFKPGMPFDLMVFVTNPDGSPAYRVP
VAVQGEDTVQSLTQGDGVAKLSINTHPSQKPLSITVRTKKQELSEAEQATRTMQ
ALPYSTVGNSNNYLHLSVLRTELRPGETLNVNFLLRMDRAHEAKIRYYTYLIMN
KGRLLKAGRQVREPGQDLVVLPLSITTDFIPSFRLVAYYTLIGASGQREVVADSV
WVDVKDSCVGSLVVKSGQSEDRQPVPGQQMTLKIEGDHGARVVLVAVDKGVF
VLNKKNKLTQSKIWDVVEKADIGCTPGSGKDYAGVFSDAGLTFTSSSGQQTAQR
AELQCPQPAARRRRSVQLTEKRMDKVGKYPKELRKCCEDGMRENPMRFSCQRR
TRFISLGEACKKVFLDCCNYITELRRQHARASHLGLARSNLDEDIIAEENIVSRSEF
PESWLWNVEDLKEPPKNGISTKLMNIFLKDSITTWEILAVSMSDKKGICVADPFE
VTVMQDFFIDLRLPYSVVRNEQVEIRAVLYNYRQNQELKVRVELLHNPAFCSLA
TTKRRHQQTVTIPPKSSLSVPYVIVPLKTGLQEVEVKAAVYHHFISDGVRKSLKV
VPEGIRMNKTVAVRTLDPERLGREGVQKEDIPPADLSDQVPDTESETRILLQGTP
VAQMTEDAVDAERLKHLIVTPSGCGEQNMIGMTPTVIAVHYLDETEQWEKFGLE
KRQGALELIKKGYTQQLAFRQPSSAFAAFVKRAPSTWLTAYVVKVFSLAVNLIAI
DSQVLCGAVKWLILEKQKPDGVFQEDAPVIHQEMIGGLRNNNEKDMALTAFVLI
SLQEAKDICEEQVNSLPGSITKAGDFLEANYMNLQRSYTVAIAGYALAQMGRLK
GPLLNKFLTTAKDKNRWEDPGKQLYNVEATSYALLALLQLKDFDFVPPVVRWL
NEQRYYGGGYGSTQATFMVFQALAQYQKDAPDHQELNLDVSLQLPSRSSKITH
RIHWESASLLRSEETKENEGFTVTAEGKGQGTLSVVTMYHAKAKDQLTCNKFDL
KVTIKPAPETEKRPQDAKNTMILEICTRYRGDQDATMSILDISMMTGFAPDTDDL
KQLANGVDRYISKYELDKAFSDRNTLIIYLDKVSHSEDDCLAFKVHQYFNVELIQ
PGAVKVYAYYNLEESCTRFYHPEKEDGKLNKLCRDELCRCAEENCFIQKSDDKV

TLEERLDKACEPGVDYVYKTRLVKVQLSNDFDEYIMAIEQTIKSGSDEVQVGQQ
RTFISPIKCREALKLEEKKHYLMWGLSSDFWGEKPNLSYIIGKDTWVEHWPEEDE
CQDEENQKQCQDLGAFTESMVVFGCPN)

- GATA4, *e.g.*, SEQ ID NO:27
(MYQSLAMAANHGPPPGAYEAGGPGAFMHGAGAASSPVYVPTPRVPSSVLGLS
YLQGGGAGSASGGASGGSSGGAASGAGPGTQQGSPGWSQAGADGAAYTPPPVS
PRFSFPGTTGSLAAAAAAAAAREAAAYSSGGGAAGAGLAGREQYGRAGFAGSY
SSPYPAYMADVGASWAAAAAASAGPFDSPVLHSLPGRANPAARHPNLDMFDDF
SEGRECVNCGAMSTPLWRRDGTGHYLCNACGLYHKMNGINRPLIKPQRRLSAS
RRVGLSCANCQTTTTTLWRRNAEGEPVCNACGLYMKLHGVPRPLAMRKEGIQT
RKRKPKNLNKSKTPAAPSGSESLPPASGASSNSSNATTSSSEEMRPIKTEPGLSSH
YGHSSSVSQTFSVSAMSGHGPSIHPVLSALKLSPQGYASPVSQSPQTSSKQDSWN
SLVLADSHGDIITA)

- p53, *e.g.*, SEQ ID NO:28
(MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDDLMLSPDDIEQWF
TEDPGPDEAPRMPEAAPPVAPAPAAPTPAAPAPAPSWPLSSSVPSQKTYQGSYGF
RLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPPGTRVRAMAIY
KQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSVV
VPYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVR
VCACPGRDRRTEEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYF
TLQIRGRERFEMFRELNEALELKDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKK
LMFKTEGPDSD)

- SP1, *e.g.*, SEQ ID NO:29
(MSDQDHSMDEMTAVVKIEKGVGGNNGGNGNGGGAFSQARSSSTGSSSSTGGG
GQESQPSPLALLAATCSRIESPNENSNNSQGPSQSGGTGELDLTATQLSQGANGW
QIISSSSGATPTSKEQSGSSTNGSNGSESSKNRTVSGGQYVVAAAPNLQNQQVLT
GLPGVMPNIQYQVIPQFQTVDGQQLQFAATGAQVQQDGSGQIQIIPGANQQIITN
RGSGGNIIAAMPNLLQQAVPLQGLANNVLSGQTQYVTNVPVALNGNITLLPVNS
VSAATLTPSSQAVTISSSGSQESGSQPVTSGTTISSASLVSSQASSSSFFTNANSYST
TTTTSNMGIMNFTTSGSSGTNSQGQTPQRVSGLQGSDALNIQQNQTSGGSLQAG

QQKEGEQNQQTQQQQILIQPQLVQGGQALQALQAAPLSGQTFTTQAISQETLQN
LQLQAVPNSGPIIIRTPTVGPNGQVSWQTLQLQNLQVQNPQAQTITLAPMQGVSL
GQTSSSNTTLTPIASAASIPAGTVTVNAAQLSSMPGLQTINLSALGTSGIQVHPIQG
LPLAIANAPGDHGAQLGLHGAGGDGIHDDTAGGEEGENSPDAQPQAGRRTRRE
ACTCPYCKDSEGRGSGDPGKKKQHICHIQGCGKVYGKTSHLRAHLRWHTGERP
FMCTWSYCGKRFTRSDELQRHKRTHTGEKKFACPECPKRFMRSDHLSKHIKTHQ
NKKGGPGVALSVGTLPLDSGAGSEGSGTATPSALITTNMVAMEAICPEGIARLAN
SGINVMQVADLQSINISGNGF)

- MEF2C, *e.g.*, SEQ ID NO:30
(MGRKKIQITRIMDERNRQVTFTKRKFGLMKKAYELSVLCDCEIALIIFNSTNKLF
QYASTDMDKVLLKYTEYNEPHESRTNSDIVETLRKKGLNGCDSPDPDADDSVGH
SPESEDKYRKINEDIDLMISRQRLCAVPPPNFEMPVSIPVSSHNSLVYSNPVSSLGN
PNLLPLAHPSLQRNSMSPGVTHRPPSAGNTGGLMGGDLTSGAGTSAGNGYGNPR
NSPGLLVSPGNLNKNMQAKSPPPMNLGMNNRKPDLRVLIPPGSKNTMPSVSEDV
DLLLNQRINNSQSAQSLATPVVSVATPTLPGQGMGGYPSAISTTYGTEYSLSSAD
LSSLSGFNTASALHLGSVTGWQQQHLHNMPPSALSQLGACTSTHLSQSSNLSLPS
TQSLNIKSEPVSPPRDRTTTPSRYPQHTRHEAGRSPVDSLSSCSSSYDGSDREDHR
NEFHSPIGLTRPSPDERESPSVKRMRLSEGWAT)

- TAX, *e.g.*, SEQ ID NO:31
(MAHFPGFGQSLLFGYPVYVFGDCVQGDWCPISGGLCSARLHRHALLATCPEHQI
TWDPIDGRVIGSALQFLIPRLPSFPTQRTSKTLKVLTPPITHTTPNIPPSFLQAMRKY
SPFRNGYMEPTLGQHLPTLSFPDPGLRPQNLYTLWGGSVVCMYLYQLSPPITWPL
LPHVIFCHPGQLGAFLTNVPYKRIEELLYKISLTTGALIILPEDCLPTTLFQPARAPV
TLTAWQNGLLPFHSTLTTPGLIWTFTDGTPMISGPCPKDGQPSLVLQSSSFIFHKF
QTKAYHPSFLLSHGLIQYSSFHSLHLLFEEYTNIPISLLFNEKEADDNDHEPQISPG
GLEPPSEKHFRETEV)

- PPARγ, *e.g.*, SEQ ID NO:32
(MGETLGDSPIDPESDSFTDTLSANISQEMTMVDTEMPFWPTNFGISSVDLSVMED
HSHSFDIKPFTTVDFSSISTPHYEDIPFTRTDPVVADYKYDLKLQEYQSAIKVEPAS
PPYYSEKTQLYNKPHEEPSNSLMAIECRVCGDKASGFHYGVHACEGCKGFFRRTI

RLKLIYDRCDLNCRIHKKSRNKCQYCRFQKCLAVGMSHNAIRFGRMPQAEKEKL
LAEISSDIDQLNPESADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPFVIY
DMNSLMMGEDKIKFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGF
VNLDLNDQVTLLKYGVHEIIYTMLASLMNKDGVLISEGQGFMTREFLKSLRKPF
GDFMEPKFEFAVKFNALELDDSDLAIFIAVIILSGDRPGLLNVKPIEDIQDNLLQAL
ELQLKLNHPESSQLFAKLLQKMTDLRQIVTEHVQLLQVIKKTETDMSLHPLLQEI
YKDLY) or

- SET9, *e.g.*, SEQ ID NO:33
(MDSDDEMVEEAVEGHLDDDGLPHGFCTVTYSSTDRFEGNFVHGEKNGRGKFFF
FDGSTLEGYYVDDALQGQGVYTYEDGGVLQGTYVDGELNGPAQEYDTDGRLIF
KGQYKDNIRHGVCWIYYPDGGSLVGEVNEDGEMTGEKIAYVYPDERTALYGKFI
DGEMIEGKLATLMSTEEGRPHFELMPGNSVYHFDKSTSSCISTNALLPDPYESER
VYVAESLISSAGEGLFSKVAVGPNTVMSFYNGVRITHQEVDSRDWALNGNTLSL
DEETVIDVPEPYNHVSKYCASLGHKANHSFTPNCIYDMFVHPRFGPIKCIRTLRA
VEADEELTVAYGYDHSPPGKSGPEAPEWYQVELKAFQATQQK), or

one or more of the transcriptional activation domains described in Chavez *et al.*, Nat Methods. 2015 Apr; 12(4): 326–328, which is hereby incorporated by reference in the entirety for any and all purposes including but not limited to activation domain polypeptides and encoding polynucleotides, Cas9 (*e.g.*, dCas9) polypeptides and encoding polynucleotides, and fusion proteins, and complexes (*e.g.*, with sgRNA) thereof.

[0059] In some cases, the CRISPR nuclease is fused to one or more affinity tags. For example, the CRISPR nuclease may be a component of a SunTag. Exemplary SunTags or SunTag components include, without limitation, one or more of the affinity tagged CRISPR nucleases or affinity tag ligands, and fusion proteins thereof, described in WO 2016/011070. In one embodiment, the CRISPR nuclease contains one or more affinity tags that are non-covalently bound to one or more ligand-transcriptional activation domain fusion proteins. In such embodiments, the transcriptional activation domain fused to the affinity tag ligand can be, *e.g.*, one or more of the transcriptional activation domains described herein, such as those of SEQ ID NOs:13-33, a transcriptional activation domain described in WO 2016/011070, or a combination or derivative thereof.

[0060] As used herein, the terms "Cas9," "Cas9 molecule," and the like, refers to a Cas9 polypeptide or a nucleic acid encoding a Cas9 polypeptide. A "Cas9 polypeptide" is a polypeptide that can form a complex with a guide RNA (gRNA) and bind to a nucleic acid target containing a target domain and, in certain embodiments, a PAM sequence. Cas9 molecules include those having a naturally occurring Cas9 polypeptide sequence and engineered, altered, or modified Cas9 polypeptides that differ, *e.g.*, by at least one amino acid residue, from a reference sequence, *e.g.*, the most similar naturally occurring Cas9 molecule. A Cas9 molecule may be a Cas9 polypeptide or a nucleic acid encoding a Cas9 polypeptide. A Cas9 molecule may be a nuclease (an enzyme that cleaves both strands of a double-stranded nucleic acid), a nickase (an enzyme that cleaves one strand of a double-stranded nucleic acid), or a catalytically inactive (or dead) Cas9 molecule. A Cas9 molecule having nuclease or nickase activity is referred to as a "catalytically active Cas9 molecule" (a "caCas9" molecule). A Cas9 molecule lacking the ability to cleave or nick target nucleic acid is referred to as a "catalytically inactive Cas9 molecule" (a "ciCas9" molecule) or a "dead Cas9" ("dCas9").

[0061] In certain embodiments, a Cas9 molecule meets one or both of the following criteria: it has at least 20, 30, 40, 50, 55, 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% homology with, or it differs by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 35, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350 or 400, amino acid residues from, the amino acid sequence of a reference sequence, e.g., a naturally occurring Cas9 molecule.

[0062] In some embodiments, the Cas9 molecule is a *S. pyogenes* Cas9 (SpCas9) or variant thereof. In some embodiments, the Cas9 molecule is a *S. aureus* Cas9 (SaCas9) or variant thereof (see, *e.g.*, FIGS 7A-11B herein). In some embodiments, the Cas9 molecule is a *Campylobacter jejuni* Cas9 (CjCas9) or variant thereof (see, *Kim et al., Nat. Comm.*, 8, 14500 (2017). In some embodiments, the Cas9 molecule is a *Neisseria meningitides* Cas9 (NmCas9) or variant thereof (see, US Patent No.: 9,074,199). In some embodiments, the Cas9 molecule is a Streptococcus *thermophilus* Cas9 (StCas9) or variant thereof (see, *e.g.*, Xu *et al., Cell Mol Life Sci.*, 72:383-99 (2014)). In some embodiments, the Cas9 molecule is a dCas9 molecule.

[0063] In certain embodiments, the Cas9 molecule is a *S. pyogenes* Cas9 variant. In certain embodiments, the Cas9 variant is the EQR variant. In certain embodiments, the Cas9 variant is

the VRER variant. In certain embodiments, the dCas9 molecule is a *S. pyogenes* Cas9 variant. In certain embodiments, the Cas9 variant is the EQR variant. In certain embodiments, the Cas9 variant is the VRER variant. In certain embodiments, a Cas9 system comprises a Cas9 molecule, *e.g.*, a Cas9 molecule described herein, e.g., the Cas9 EQR variant or the Cas9 VRER variant.

[0064] In certain embodiments, the Cas9 molecule is a *S. aureus* Cas9 variant. In certain embodiments, the Cas9 variant is the KKH (E782K/N968K/R1015H) variant (see, e.g., Kleinstiver *et al.*, Nature 523, 481–485 (23 July 2015); and Leenay *et al.* Molecular Cell, Vol. 62, Issue 1, 2016, p. 137), the entire contents of which are expressly incorporated herein by reference and especially with regard to Cas (*e.g.*, Cas9) variants such as those having altered PAM specificities). In certain embodiments, the Cas9 variant is the E782K/K929R/R1015H variant (see, *e.g.*, Kleinstiver 2015). In certain embodiments, the Cas9 variant is the E782K/K929R/N968K/R1015H variant (see, *e.g.*, Kleinstiver 2015). In certain embodiments the Cas9 variant comprises one or more mutations in one of the following residues: E782, K929, N968, R1015. In certain embodiments the Cas9 variant comprises one or more of the following mutations: E782K, K929R, N968K, R1015H and R1015Q (see, e.g., Kleinstiver 2015). In certain embodiments, a Cas9 system comprises a Cas9 molecule, *e.g.*, a Cas9 molecule described herein, *e.g.*, the Cas9 KKH variant.

[0065] As used herein, the terms "Cpf1," "Cpf1 molecule," and the like, refers to a Cpf1 polypeptide or a nucleic acid encoding a Cpf1 polypeptide. A "Cpf1 polypeptide" is a polypeptide that can form a complex with a guide RNA (gRNA) and bind to a nucleic acid target containing a target domain and, in certain embodiments, a PAM sequence. Cpf1 molecules include those having a naturally occurring Cpf1 polypeptide sequence and engineered, altered, or modified Cpf1 polypeptides that differ, *e.g.*, by at least one amino acid residue, from a reference sequence, *e.g.*, the most similar naturally occurring Cpf1 molecule. A Cpf1 molecule may be a Cpf1 polypeptide or a nucleic acid encoding a Cpf1 polypeptide. Examplary Cpf1 polypeptides include those isolated from *Prevotella, Francisella* novicida (FnCpf1), *Lachnospiraceae bacterium* (LbCpf1) and *Acidaminococcus sp. (AsCpf1) (see, e.g.*, Tóth *et al., Biology Direct*, 11:46 (2016).

[0066] In certain embodiments, a Cpf1 molecule meets one or both of the following criteria: it has at least 20, 30, 40, 50, 55, 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93,

94, 95, 96, 97, 98, 99, or 100% homology with, or it differs by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 35, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350 or 400, amino acid residues from, the amino acid sequence of a reference sequence, e.g., a naturally occurring Cpf1 molecule.

[0067] As used herein, the term "gRNA molecule" or "gRNA" refers to a guide RNA which is capable of targeting a CRISPR nuclease to a target nucleic acid. In one embodiment, the term "gRNA molecule" refers to a guide ribonucleic acid. In another embodiment, the term "gRNA molecule" refers to a nucleic acid encoding a gRNA. In one embodiment, a gRNA molecule is non-naturally occurring. In one embodiment, a gRNA molecule is a synthetic gRNA molecule.

[0068] The guide RNA can be a scaffold RNA that binds to one or more protein or nucleic acid ligands (scaffold RNA ligands). The ligands can be fused or otherwise covalently or non-covalently linked to transcriptional activation domains. In an alternative embodiment, the scaffold RNA is not a guide RNA in that it does not specifically associate with a CRISPR nuclease. Exemplary scaffold RNAs, and CRISPR nuclease/scaffold RNA complexes, and methods of making and using such, are described in, *e.g.*, WO 2016/054106 (describing CRISPR-associating and CRISPR independent scaffold RNAs) and Zhang *et al.*, Scientific Reports 5, Article No. 16277 (2015); Konermann *et al.*, 2015, Nature 517:583-8 (describing CRISPR/gRNA-directed synergistic activation mediators (SAM)).

[0069] "Subject," as used herein, may mean either a human or non-human animal. The term includes, but is not limited to, mammals (e.g., humans, other primates, pigs, rodents (e.g., mice and rats or hamsters), rabbits, guinea pigs, cows, horses, cats, dogs, sheep, and goats). In an embodiment, the subject is a human. In another embodiment, the subject is poultry. In another embodiment, the subject is piscine. In certain embodiments, the subject is a human, and in certain of these embodiments the human is an infant, child, young adult, or adult.

[0070] As used herein, the terms "target nucleic acid" or "target gene" refer to a nucleic acid which is being targeted for binding, *e.g.*, by a CRISPR nuclease in complex with a guide RNA, a guide-RNA, or a scaffold RNA. In certain embodiments, a target nucleic acid comprises one gene, or a promoter or enhancer region operably linked to one gene. In certain embodiments, a target nucleic acid may comprise one or more genes, *e.g.*, two genes, three genes, four genes, or five genes, or promoters or enhancer regions operably linked to one or more genes. In one

embodiment, a target nucleic acid may comprise a promoter region, or control region, of a gene. In one embodiment, a target nucleic acid may comprise an intron of a gene. In another embodiment, a target nucleic acid may comprise an exon of a gene. In one embodiment, a target nucleic acid may comprise a coding region of gene. In one embodiment, a target nucleic acid may comprise a non-coding region of a gene. In some embodiments, the target nucleic acid is a control region, promoter, enhancer, intron, exon, transcription start site, coding region, or non-coding region of a gene listed in Table 1 herein.

[0071] In some embodiments, the target nucleic acid is a control region, promoter, enhancer, intron, exon, transcription start site, coding region, or non-coding region of a gene in the same pathway as a gene listed in Table 1 herein. The target nucleic acid can, *e.g.*, be a control region, promoter, enhancer, intron, exon, transcription start site, coding region, or non-coding region of a gene upstream and in the same pathway as a gene listed in Table 1 herein. Additionally, where two or more genes or positions are targeted, or alternatively, the target nucleic acid can, *e.g.*, be a control region, promoter, enhancer, intron, exon, transcription start site, coding region, or non-coding region of a gene downstream and in the same pathway as a gene listed in Table 1 herein. Additionally, where two or more genes or positions are targeted, or alternatively, the target nucleic acid can, *e.g.*, be a control region, promoter, enhancer, intron, exon, transcription start site, coding region, or non-coding region of a gene in a parallel pathway as a gene listed in Table 1 herein. Exemplary genes in the same pathway or a parallel pathway as one or more of the genes listed in Table 1 are described *e.g.*, in the KEGG pathway database (available at www.genome.jp/kegg/pathway.html).

[0072] "Target position" as used herein, refers to a site on a target nucleic acid that is hybridized to a guide RNA (*e.g.*, in complex with a CRISPR nuclease) or scaffold RNA. Optimized target positions include, without limitation, one or more target positions optimized for transcriptional activation that are described in WO 2016/011080.

[0073] "Episomal vector" or "episomally propagating vector" refers to a plasmid or viral vector that persists or propagates in a mammalian cell as an episomal element. Episomal vectors described herein can encode one or more components (*e.g.*, CRISPR nuclease, guide RNA, zinc finger nuclease, TALEN, TAL effector, scaffold RNA, transcriptional activator, affinity element, or combination thereof) for treatment of a disease or condition by transcriptional activation (*e.g.*,

a disease or condition of Table 1). Episomal vectors include, but are not limited to, Adeno-associated virus (AAV) vectors, and Epstein-barr virus (EBV) vectors. Suitable AAV vectors and methods for making and using such AAV vectors, *e.g.*, for delivering the vectors into target cells are described in Samulski Ret al. (1987), J. Virol. 61: 3096-3101; Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); Fisher K J et al. (1996), J. Virol, 70: 520-532; Samulski Ret al. (1989), J. Virol. 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; U.S. Pat. No. 5,436,146; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641, the entire disclosures of which are herein incorporated by reference.

[0074] As used herein, the term "Zinc Finger Nuclease" refers to a zinc finger DNA binding protein (or zinc finger DNA binding domain within a larger protein) that binds DNA in a sequence-specific manner through one or more zinc fingers, which are regions of amino acid sequence within the zinc finger binding domain whose structure is stabilized through coordination of a zinc ion. The term zinc finger DNA binding protein is often abbreviated as zinc finger nuclease or ZFN.

[0075] As used herein, the term "transcription activator-like effector nuclease" refers to a protein, that includes a transcription activator-like effector DNA-binding domain fused to a DNA cleavage domain, that binds DNA in a sequence-specific manner. The term transcription activator-like effector nuclease is often abbreviated to TALEN.

DETAILED DESCRIPTION OF THE INVENTION

Introduction

[0076] Described herein are methods and compositions for treating a disease in a mammalian subject associated with, exacerbated by, or caused by reduced transcription of a gene, reduced amount of a gene product, or reduced activity of a gene product by increasing transcription of a target gene. Such methods and compositions can be useful, *e.g.*, for treating a haploinsufficiency disease in the subject. Haploinsufficiency diseases that can be treated by the methods and compositions described herein include, without limitation, one or more of the diseases listed in Table 1. Table 1 provides the Entrez Gene ID (column 2) from the national center for bioinformatics (NCBI) and corresponding gene symbol (column 1) provided by the human

genome nomenclature committee (HGNC), a pubmed ID (PMID) citation to a supporting reference (column 4), and a brief description of the associated disorder (column 5). The table is adapted from Supplementary Table 1 of Dang *et al.*, European Journal of Human Genetics (2008) 16, 1350-57and the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) and ClinGen (https://www.clinicalgenome.org) databases.

Nucleases

[0077] In some embodiments of the methods described herein, a host cell is contacted with one or more nucleases. In some embodiments, the nuclease is a endonuclease, site-specific recombinase, transposase, topoisomerase, zinc finger nuclease, TALEN, and includes modified derivatives and variants thereof.

[0078] In some embodiments, a nuclease is capable of targeting a designated nucleotide or region within the target site. In some embodiments, the nuclease is capable of targeting a region positioned between the 5' and 3' regions of the target site. In another embodiment, the nuclease is capable of targeting a region positioned upstream or downstream of the 5' and 3' regions of the target site (e.g., upstream or downstream of the transcription start site (TSS)). A recognition sequence is a polynucleotide sequence that is specifically recognized and/or bound by the nuclease. The length of the recognition site sequence can vary, and includes, for example, nucleotide sequences that are at least 10, 12, 14, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70 or more nucleotides in length. In some embodiments, the recognition sequence is palindromic, *i.e.*, the sequence on one DNA strand reads the same in the opposite direction on the complementary DNA strand. In some embodiments, the target site of the nuclease is within the recognition sequence.

Zinc Finger Nuclease

[0079] In some embodiments, the nuclease is a zinc-finger nuclease (ZFN). ZFNs typically comprise a zinc finger DNA binding domain and a nuclease domain. Generally, ZFNs include two zinc finger arrays (ZFAs), each of which is fused to a single subunit of a non-specific endonuclease, such as the nuclease domain from the FokI enzyme, which becomes active upon dimerization. Typically, a single ZFA consists of 3 or 4 zinc finger domains, each of which is designed to recognize a specific nucleotide triplet (GGC, GAT, *etc.*). A ZFN composed of two

"3-finger" ZFAs is therefore capable of recognizing an 18 base pair target site (*i.e.*, recognition sequence); an 18 base pair recognition sequence is generally unique, even within large genomes such as those of humans and plants. By directing the co-localization and dimerization of the two FokI nuclease monomers, ZFNs generate a functional site-specific endonuclease that can target a particular locus (*e.g.*, gene, promotor or enhancer).

[0080] Zinc-finger nucleases useful in the methods disclosed herein include those that are known and ZFN that are engineered to have specificity for one or more target sites described herein (*e.g.*, promotor or enhancer nucleotide sequence). Zinc finger domains are amenable for designing polypeptides which specifically bind a selected polynucleotide recognition sequence within a target site of the host cell genome. ZFN can comprise an engineered DNA-binding zinc finger domain linked to a non-specific endonuclease domain, for example, a nuclease domain from a Type IIs endonuclease such as HO or FokI. In some examples, a zinc finger DNA binding domain can be fused to a site-specific recombinase, transposase, or a derivative thereof that retains DNA nicking and/or cleaving activity.

[0081] In a preferred embodiment, additional functionalities can be fused to the zinc-finger binding domain, including but not limited to, transcriptional activator domains (such as VP16, VP48, VP64, VP160 and the like) or transcription repressor domains (such as KRAB). In one embodiment, the zinc finger nuclease is engineered such that the zinc finger nuclease comprises a transcriptional activator domain selected from VP16, VP48, VP64 or VP160. In one embodiment, the zinc finger nuclease is engineered such that the zinc finger nuclease comprises a transcriptional activator domain selected from HSF1, VP16, VP64, p65, RTA, MyoD1, SET7, VPR, histone acetyltransferase p300, TET1 hydroxylase catalytic domain, LSD1, CIB1, AD2, CR3, GATA4, p53, SP1, MEF2C, TAX, PPAR-gamma, and SET9. For example, engineered zinc finger transcriptional activator that interact with a promoter region of the gamma-globulin gene was shown to enhance fetal hemoglobin production in primer adult erythroblasts (Wilber *et al.*, *Blood*, 115(15):3033-3041). Other polydactyl zinc-finger transcription factors are also known in the art, including those disclosed in Beerli and Barbas (see, *Nature Technology*, (2002) 20:135-141).

[0082] Each zinc finger domain recognizes three consecutive base pairs in the target DNA. For example, a three finger domain recognizes a sequence of nine contiguous nucleotides, with a

dimerization requirement of the nuclease, two sets of zinc finger triplets are used to bind a 18 nucleotide recognition sequence. Useful zinc finger modules include those that recognize various GNN and ANN triplets (Dreier *et al.,* (2001) *J Biol Chem* 276:29466-78; Dreier et al., (2000) *J Mol Biol* 303:489-502; Liu *et al.,* (2002) *J Biol Chem* 277:3850-6), as well as those that recognize various CNN or TNN triplets (Dreier et al., (2005*) J Biol Chem* 280:35588-97; Jamieson *et al.,* (2003) *Nature Rev Drug Discovery* 2:361-8). See also, Durai *et al.,* (2005) *Nucleic Acids Res* 33:5978-90; Segal, (2002) *Methods* 26:76-83; Porteus and Carroll, (2005) *Nat Biotechnology* 23:967-73; Pabo *et al.,* (2001) *Ann Rev Biochem* 70:313-40; Wolfe *et al.,* (2000) *Ann Rev Biophys Biomol Struct* 29:183-212; Segal and Barbas (2001) *Curr Opin Biotechnol* 12:632-7; Segal *et al.,* (2003) *Biochemistry* 42:2137-48; Beerli and Barbas, (2002) *Nat Biotechnol* 20:135-41; Carroll *et al.,* (2006) *Nature Protocols* 1:1329; Ordiz *et al.,* (2002) *Proc Natl Acad Sci USA* 99:13290-5; Guan *et al.*, (2002) *Proc Natl Acad Sci USA* 99:13296-301; WO2002099084; WO00/42219; WO02/42459; WO2003062455; US20030059767; US Patent Application Publication Number 2003/0108880; U.S. Pat. Nos. 6,140,466, 6,511,808 and 6,453,242. Useful zinc-finger nucleases also include those described in WO03/080809; WO05/014791; WO05/084190; WO08/021207; WO09/042186; WO09/054985; and WO10/065123.

[0083] In some embodiments, a ZFN comprises a fusion protein having a cleavage domain of a Type IIS restriction endonuclease fused to an engineered zinc finger binding domain, wherein the binding domain further comprises one or more transcriptional activators. In some embodiments, the type IIS restriction endonuclease is selected from a HO endonuclease or a FokI endonuclease. In some embodiments, the zinc finger binding domain comprises 3, 4, 5 or 6 zinc fingers. In another embodiment, the zinc finger binding domain specifically binds to a recognition sequence corresponding to a promoter or enhancer disclosed herein (*e.g.,* SIM1, MC4R, PKD1, SETD5, THUMPD3, SCN2A and PAX6 promotor or enhancer). In one embodiment, the one or more transcriptional activators is selected from VP16, VP48, VP64, or VP160. Generally, the DNA-binding domain of a ZFN contains between 3 and 6 individual zinc finger repeats and can recognize between 9 and 18 contiguous nucleotides. Each ZFN can be designed to target a specific target site in the host cell genome, *e.g.,* a promotor sequence, an enhancer sequence, or exon/intron within a gene.

TALENs

[0084] In some embodiments of the methods, the nuclease is a TALEN. TAL effectors (TALEs) are proteins secreted by *Xanthomonas* bacteria and play an important role in disease or triggering defense mechanisms, by binding host DNA and activating effector-specific host genes. see, *e.g.,* Gu et al. (2005) *Nature* 435:1122-5; Yang *et al.,* (2006) *Proc. Natl. Acad. Sci. USA* 103:10503-8; Kay *et al.,* (2007) *Science* 318:648-51; Sugio *et al.,* (2007) *Proc. Natl. Acad. Sci. USA* 104:10720-5; Romer *et al.,* (2007) *Science* 318:645-8; Boch *et al.,* (2009) *Science* 326(5959):1509-12; and Moscou and Bogdanove, (2009) 326(5959):1501. A TALEN comprises a TAL effector DNA-binding domain fused to a DNA cleavage domain. The DNA binding domain interacts with DNA in a sequence-specific manner through one or more tandem repeat domains. The repeated sequence typically comprises 33-34 highly conserved amino acids with divergent 12^{th} and 13^{th} amino acids. These two positions, referred to as the Repeat Variable Diresidue (RVD) are highly variable and show a strong correlation with specific nucleotide recognition (Boch *et al.,* (2009) *Science* 326(5959):1509-12; and Moscou and Bogdanove, (2009) 326(5959):1501). This relationship between amino acid sequence and DNA recognition sequence has allowed for the engineering of specific DNA-binding domains by selecting a combination of repeat segments containing the appropriate RVDs.

[0085] The TAL-effector DNA binding domain can be engineered to bind to a target DNA sequence and fused to a nuclease domain, *e.g.,* a Type IIS restriction endonuclease, such as FokI (see *e.g.,* Kim et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:1156-1160). In some embodiments, the nuclease domain can comprises one or more mutations (*e.g.,* FokI variants) that improve cleavage specificity (see, *Doyon et al., (2011) Nature Methods, 8 (1): 74–9)* and cleavage activity (*Guo et al., (2010) Journal of Molecular Biology, 400 (1): 96–107).* Other useful endonucleases that can be used as the nuclease domain include, but are not limited to, HhaI, HindIII, Nod, BbvCI, EcoRI, BglI, and AlwI. In some embodiments, the TALEN can comprise a TAL effector DNA binding domain comprising a plurality of TAL effector repeat sequences that bind to a specific nucleotide sequence (i.e., recognition sequence) in the target DNA. While not to be construed as limiting, TALENs useful for the methods provided herein include those described in WO10/079430 and U.S. Patent Application Publication No. 2011/0145940.

[0086] In some embodiments, the TAL effector DNA binding domain can comprise 10 or more DNA binding repeats, and preferably 15 or more DNA binding repeats. In some embodiments, each DNA binding repeat comprises a RVD that determines recognition of a base pair in the target DNA, and wherein each DNA binding repeat is responsible for recognizing one base pair in the target DNA. In some embodiments, the RVD comprises one or more of: HD for recognizing C; NG for recognizing T; NI for recognizing A; NN for recognizing G or A; NS for recognizing A or C or G or T; N* for recognizing C or T, where * represents a gap in the second position of the RVD; HG for recognizing T; H* for recognizing T, where * represents a gap in the second position of the RVD; IG for recognizing T; NK for recognizing G; HA for recognizing C; ND for recognizing C; HI for recognizing C; HN for recognizing G; NA for recognizing G; SN for recognizing G or A; and YG for recognizing T.

[0087] In a preferred embodiment, the TALEN is engineered such that the TAL effector comprises one or more transcriptional activator domains (*e.g.*, VP16, VP48, VP64 or VP160). For example, engineered TAL effectors having a transcriptional activator domain at the c-terminus of the TAL effector were shown to modulate transcription of Sox2 and Klf4 genes in human 293FT cells (Zhang *et al.*, *Nature Biotechnology*, 29(2):149-153 (2011). Other TAL effector transcription factors (TALE-TFs) are also known in the art, including those disclosed in Perez-Pinera *et al.*, (*Nature Methods*, (2013) 10(3):239-242) that demonstrated modulation of IL1RN, KLK3, CEACAM5 and ERBB2 genes in human 293T cells using TALE-TFs. In some embodiments, the one or more transcriptional activator domains are located adjacent to the nuclear localization signal (NLS) present in the C-terminus of the TAL effector. In another embodiment, the TALE-TFs can bind nearby sites upstream or downstream of the transcriptional start site (TSS) for a target gene. In one embodiment, the TAL effector comprises a transcriptional activator domain selected from VP16, VP48, VP64 or VP160. In another embodiment, the TAL effector comprises a transcriptional activator domain selected from HSF1, VP16, VP64, p65, RTA, MyoD1, SET7, VPR, histone acetyltransferase p300, TET1 hydroxylase catalytic domain, LSD1, CIB1, AD2, CR3, GATA4, p53, SP1, MEF2C, TAX, PPAR-gamma, and SET9.

[0088] In some embodiments, the TALEN comprises a TAL effector DNA-binding domain fused to a DNA cleavage domain, wherein the TAL effector comprises a transcriptional

activator. In some embodiments, the DNA cleavage domain is of a Type IIS restriction endonuclease selected from a HO endonuclease or a FokI endonuclease. In some embodiments, the TAL effector DNA-binding domain specifically binds to a recognition sequence corresponding to a promoter region or enhancer region disclosed herein (*e.g.*, SIM1, MC4R, PKD1, SETD5, THUMPD3, SCN2A and PAX6 promotor or enhancer). Generally, the DNA-binding domain of a TALEN is designed to target a specific target site in the host cell, *e.g.*, a promotor sequence or an enhancer sequence.

[0089] In some embodiments, the target site for the zinc finger nuclease or TALEN is endogenous to the host cell, such as a native locus in the host cell genome. In some embodiments, the target site is selected according to the type of nuclease to be utilized in the method. If the nuclease to be utilized is a zinc finger nuclease, optimal target sites may be selected using a number of publicly available online resources. See, *e.g.*, Reyon et al., *BMC Genomics* 12:83 (2011), which is hereby incorporated by reference in its entirety. Publicly available methods for engineering zinc finger nucleases include: (1) Context-dependent Assembly (CoDA), (2) Oligomerized Pool Engineering (OPEN), (3) Modular Assembly, (4) ZiFiT (internet-accessible software for the design of engineered zinc finger arrays), (5) ZiFDB (internet-accessible database of zinc fingers and engineered zinc finger arrays), and (6) ZFNGenome. For example, OPEN is a publicly available protocol for engineering zinc finger arrays with high specificity and *in vivo* functionality, and has been successfully used to generate ZFNs that function efficiently in plants, zebrafish, and human somatic and pluripotent stem cells. OPEN is a selection-based method in which a pre-constructed randomized pool of candidate ZFAs is screened to identify those with high affinity and specificity for a desired target sequence. Additionally, ZFNGenome is a GBrowse-based tool for identifying and visualizing potential target sites for OPEN-generated ZFNs. ZFNGenome provides a compendium of potential ZFN target sites in sequenced and annotated genomes of model organisms. ZFNGenome includes more than 11 million potential ZFN target sites, mapped within the fully sequenced genomes of seven model organisms; S. *cerevisiae, C. reinhardtii, A. thaliana, D. melanogaster, D. rerio, C. elegans,* and *H. sapiens*. ZFNGenome provides information about each potential ZFN target site, including its chromosomal location and position relative to transcription initiation site(s). Users can query ZFNGenome using several different criteria (*e.g.*, gene ID, transcript ID, target site sequence).

[0090] In some embodiments, if the nuclease is a TALEN, optimal target sites may be selected in accordance with the methods described by Sanjana et al., *Nature Protocols*, 7:171-192 (2012), which is hereby incorporated by reference in its entirety. TALENs function as dimers, and a pair of TALENs, referred to as the left and right TALENs, target sequences on opposite strands of DNA. TALENs are engineered as a fusion of the TALE DNA-binding domain and a monomeric FokI catalytic domain. To facilitate FokI dimerization, the left and right TALEN target sites are generally selected with a spacing of approximately 14-20 bases.

[0091] In some embodiments, the one or more nucleases useful for the methods described herein are provided, *e.g.,* delivered into the host cell as a purified protein. In some embodiments, the one or more nucleases are provided via polynucleotide(s) comprising a nucleic acid encoding the nuclease. In another embodiment, the one or more nucleases can be introduced into the host cell as purified RNA which can be directly translated in the host cell nucleus. In a preferred embodiment, the polynucleotide comprising a nucleic acid encoding the nuclease comprises an expression vector that allows for the expression of the nuclease within a host cell. Suitable expression vectors include episomal vectors.

[0092] In some embodiments, where the nuclease functions as a dimer requiring the separate expression of each monomer, *e.g.,* zinc finger nucleases and TALENs, each monomer of the dimer may be expressed from the same episomal vector or from different episomal vectors. In another embodiment, where multiple nucleases are introduced to the cell to introduce double-strand breaks at different target sites, the nucleases can be encoded on a single episomal vector or on separate episomal vectors.

[0093] In one aspect, the present invention provides a method of treating a haploinsufficiency disease in a mammalian subject, the method comprising contacting a cell of the subject with a composition comprising a zinc finger nuclease or TALEN that, under conditions present in a nucleus of the cell, the zinc finger nuclease or TALEN specifically hybridizes to a promoter region or an enhancer region; wherein the contacting forms a complex comprising the DNA binding domain of the zinc finger nuclease or TALEN, and the promoter region or enhancer region, wherein the complex activates transcription of the wild-type copy of the haploinsufficient gene in an amount and for a duration sufficient to treat the haploinsufficiency disease in the

subject. In some embodiments, the promoter or enhancer region corresponds to a promoter or enhancer region (i.e., control region) of any of the genes listed in Table 1.

[0094] In some embodiments, the contacting comprises contacting the cell with an episomal vector encoding the zinc finger nuclease or TALEN. In some embodiments, the episomal vector(s) are non-integrating. In some embodiments, the zinc finger nuclease or TALEN has been modified to comprises one or more transcriptional activation domains. In one embodiment, the one or more transcriptional activation domains is selected from the group consisting of HSF1, VP16, VP64, p65, MyoD1, RTA, SET7/9, VPR, histone acetyltransferase p300, an hydroxylase catalytic domain of a TET family protein (e.g., TET1 hydroxylase catalytic domain), LSD1, CIB1, AD2, CR3, EKLF1, GATA4, PRVIE, p53, SP1, MEF2C, TAX, and PPARγ. In some embodiments, the transcriptional activaton domain is VP64. In some embodiments, the haploinsufficient gene is SIM1, Leptin, Leptin receptor, MC4R, SCN2A, SETD5, PAX6, PKD1, MC3R, POMC, STAT3, STAT5, SOCS3, GHR, NPY, NPY1R, NPY2R, NPY5R, PYY, AMPK (PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1, PRKAG2, PRKAG3), OXT, JAK2, SHP2, NOS3, NROB2, BRS3, CARTPT, FABP4, HTR2C, IL6, NHLH2, NMU, NPB, NPBWRI, PNPLA2, UCP3, ADIPOQ, APOA5, ARNT2, ASIP, C1QTNF2, C3AR1, CCK, CPT1B, CSF2, DGAT1, DGAT2, GHRL, GHSR, HSD11B1, HTR7, INSIG1, INSIG2, LIPC, NMURI, NMUR2, NPBWR2, NTS, PPARGC1A, PPY, RETN, SIRT1, TGFBR2, WDTC1, or FOXO1.

Table 1: Genes Associated With Haploinsufficiency Diseases

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
TP73	7161	1	11454718	prostate hyperplasia and prostate cancer
DFFB	1677	1	16156899	oligodendroglioma development
KCNAB2	8514	1	11580756	characteristic craniofacial abnormalities, mental retardation, and epilepsy with 1p36 deletion syndrome
CHD5	26038	1	-	monosomy 1p36 syndrome
CAMTA1	23261	1	15709179	tumors development
PINK1	65018	1	15349860	sporadic early-onset parkinsonism

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
SAM68	10657	1	17927519	mammary tumor onset and tumor multiplicity
KCNQ4	9132	1	-	DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL 2
GLUT1	6513	1	12029447, 11477212, 11136715, 16497725	Facilitated glucose transporter protein type 1 (GLUT1) deficiency syndrome
MYH	4595	1	16292541	hepatocellular carcinoma and cholangiocarcinom
FOXE3	2301	1	11980846	anterior segment dysgenesis similar to Peters' anomaly
HUD	1996	1	16278682	poor prognosis
INK4C	1031	1	16260494	medulloblastoma formation
NFIA	4774	1	17530927	Complex central nervous system (CNS) malformations and urinary tract defects
CCN1	3491	1	17023674	delayed formation of the ventricular septum in the embryo and persistent ostium primum atrial septal defects
ABCA4	24	1	-	Stargardt disease, retinitis pigmentosa-19, and macular degeneration age-related 2
WNT2B	7482	1	17351355	mental retardation, short stature and colobomata
ADAR	103	1	16536805	dyschromatosis symmetrica hereditaria
ATP1A2	477	1	-	familial hemiplegic migraine type 2
MPZ	4359	1	-	neurologic diseases, including CHN, DSS, and CMT1B
MYOC	4653	1	-	hereditary juvenile-onset open-angle glaucoma
HRPT2	79577	1	16458039	Ossifying fibroma (progressive enlargement of the affected jaw)
LRH-1	2494	1	17670946, 15684064	inflammatory bowel disease
IRF6	3664	1	-	van der Woude syndrome and popliteal pterygium syndrome

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
PROX1	5629	1	-	Lymphatic vascular defects, adult-onset obesity
TP53BP2	7159	1	-	no suppression of tumor growth
NLRP3	114548	1	-	CINCA syndrome
ID2	3398	2	15569159	Congenital hydronephrosis
MYCN	4613	2	15821734	reduced brain size and intestinal atresias in Feingold syndrome
GCKR	2646	2	9570959	one form of maturity onset diabetes of the young
SPAST	6683	2	-	SPASTIC PARAPLEGIA 4
MSH6	2956	2	10751599	limitation of mismatch repair
FSHR	2492	2	14502087	degenerative changes in the central nervous system
SPR	6697	2	15241655	dopa-responsive dystonia
PAX8	7849	2	-	congenital hypothyroidism
SMADIP1	9839	2	11595972, 16688751	syndromic Hirschsprung disease
RPRM	56475	2	15592418	tumorigenesis, no suppression of tumor growth
SCN1A	6323	2	16865694, 16075041	Severe myoclonic epilepsy of infancy (SMEI) or Dravet syndrome
HOXD13	3239	2	12900906	foot malformations
COL3A1	1281	2	-	Ehlers-Danlos syndrome type IV, and with aortic and arterial aneurysms
SLC40A1	30061	2	16135412	ferroportin disease
SATB2	23314	2	-	craniofacial dysmorphologies, cleft palate
SUMO1	7341	2	17606301, 16990542	nonsyndromic cleft lip and palate
BMPR2	659	2	11115378	primary pulmonary hypertension
XRCC5	7520	2	16325483	retarded growth, increased radiosensitivity, elevated p53 levels and shortened telomeres
PAX3	5077	2	12070244, 9731536	developmental delay and autism

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
STK25	10494	2	15521982	mild-to-moderate mental retardation with an Albright hereditary osteodystrophy-like phenotype
CHL1	10752	3	-	3p deletion (3p-) syndrome
SRGAP3	9901	3	12195014	severe mental retardation
VHL	7428	3	16061637	increased lung cancer susceptibility
GHRL	51738	3	-	GHRELIN POLYMORPHISM
PPARG	5468	3	15073042	susceptibility to mammary, ovarian and skin carcinogenesis
SRG3	6599	3	17255092	proteasomal degradation
RASSF1A	11186	3	11585766	pathogenesis of a variety of cancers, no suppression of tumor growth
TKT	7086	3	-	reduced adipose tissue and female fertility
MITF	4286	3	10952390, 9170159	Waardenburg syndrome type 2
FOXP1	27086	3	-	tumors development
ROBO1	6091	3	-	predispose to dyslexia
DIRC2	84925	3	-	onset of tumor growth
ATP2C1	27032	3	15811312, 17597066	orthodisease, skin disorder
FOXL2	668	3	11468277	blepharophimosis syndrome associated with ovarian dysfunction
ATR	545	3	15282542	mismatch repair-deficient
SI	6476	3	-	SUCRASE-ISOMALTASE DEFICIENCY, CONGENITAL
TERC	7012	3	16284252, 15326392	Autosomal dominant dyskeratosis congenita (AD DC), a rare inherited bone marrow failure syndrome
SOX2	6657	3	16529618, 15503273	hippocampal malformations and epilepsy
OPA1	4976	3	16735988, 11017080	optic atrophy
TFRC	7037	3	-	stressed erythropoiesis and neurologic abnormalities

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
FGFR3	2261	4	9199352	a variety of skeletal dysplasias, including the most common genetic form of dwarfism, achondroplasia
LETM1	3954	4	16719275	Wolf Hirshhorn syndrome
SH3BP2	6452	4	-	Wolf-Hirschhorn syndrome
MSX1	4487	4	14630905	oligodontia
RBPJ	3516	4	-	embryonic lethality and formation of arteriovenous malformations
PHOX2B	8929	4	-	predispose to Hirschsprung disease
ENAM	10117	4	15649948	Amelogenesis imperfecta (inherited defects of dental enamel formation)
MAPK10	5602	4	-	epileptic encephalopathy of the Lennox-Gaustaut type
PKD2	5311	4	16720597, 10615132	Autosomal dominant polycystic kidney disease
SNCA	6622	4	12477695	familial Parkinson's disease
RIEG	5308	4	9480756	Rieger syndrome (RIEG) characterized by malformations of the anterior segment of the eye, failure of the periumbilical skin to involute, and dental hypoplasia
ANK2	287	4	-	arrhythmia
MAD2L1	4085	4	17038523	optimal hematopoiesis
PLK4	10733	4	16025114	mitotic infidelity and carcinogenesis
FBXW7	55294	4	-	cancer (breast, ovary) tumors development
TERT	7015	5	-	DYSKERATOSIS CONGENITA
SEMA5A	9037	5	9464278	abnormal brain development
GDNF	2668	5	11774071	complex human diseases (Hirschsprung-like intestinal obstruction and early-onset lethality)
FGF10	2255	5	16476029, 15654336	craniofacial development and developmental disorders

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
PIK3R1	5295	5	10829070	insulin resistance
APC	324	5	14691304	familial adenomatous polyposis
RAD50	10111	5	16474176	hereditary breast cancer susceptibility associated with genomic instability
SMAD5	4090	5	12064918	secondary myelodysplasias and acute myeloid leukemias
EGR1	1958	5	17420284	development of myeloid disorders
TCOF1	6949	5	17552945, 16465596, 15930015, 15249688	depletion of neural crest cell precursors, Treacher Collins syndrome
NPM1	4869	5	16341035, 16007073	myelodysplasias and leukemias
NKX2-5	1482	5	16470726, 10398271, 15368344	microcephaly and congenital heart disease
MSX2	4488	5	10742104	pleiotropic defects in bone growth and ectodermal organ formation
NSD1	64324	5	16970856, 16547423, 15720303, 15640245, 15539801, 14631206, 14517949, 12687502, 12676901, 11896389	Sotos syndrome
FOXC1	2296	6	14564054, 11170889	Axenfeld-Rieger anomaly of the anterior eye chamber
DSP	1832	6	11841538, 11476106, 10594734, 17475244	skin fragility/woolly hair syndrome; disruption of tissue structure, integrity and changes in keratinocyte proliferation
EEF1E1	9521	6	-	no suppression of tumor growth
TNXA	7146	6	15733269	Ehlers-Danlos syndrome
TNX	7148	6	15733269	Elastic fiber abnormalities in hypermobility type Ehlers-Danlos syndrome
HMGA1	3159	6	-	insulin resistance and diabetes
RUNX2	860	6	16270353, 16187316, 15952089, 15566413, 10204840, 9690033, 9207800	cleidocranial dysplasia

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
CD2AP	23607	6	12764198	glomerular disease susceptibility
ELOVL4	6785	6	17311087, 17254625	defective skin permeability barrier function and neonatal lethality
NT5E	4907	6	12805562	Neuropathy target esterase deficiency
SIM1	6492	6	16728530, 10587584	impaired melanocortin-mediated anorexia and activation of paraventricular nucleus neurons
COL10A1	1300	6	-	Schmid type metaphyseal chondrodysplasia and Japanese type spondylometaphyseal dysplasia
PARK2	5071	6	-	PARKINSON DISEASE 2
TWIST1	7291	7	16540516, 16237669, 17003487, 15829502, 11854168	coronal synostosis
GLI3	2737	7	15739154, 14608643, 9054938	Greig cephalopolysyndactyly and Pallister-Hall syndromes
GCK	2645	7	-	non-insulin dependent diabetes mellitus (NIDDM), maturity onset diabetes of the young, type 2 (MODY2) and persistent hyperinsulinemic hypoglycemia of infancy (PHHI)
FKBP6	8468	7	15770126	Williams-Beuren syndrome
ELN	2006	7	14556246, 10198167, 16820942, 16784071, 16476938, 12016585, 11735026, 10942104, 10885576, 10780788	cardiovascular disease and connective tissue abnormalities
LIMK1	3984	7	9637430	Williams syndrome (WS), a neurodevelopmental disorder
RFC2	5982	7	-	growth deficiency as well as developmental disturbances in Williams syndrome
GTF3	9569	7	10573005	abnormal muscle fatiguability
GTF2I	2969	7	-	Williams-Beuren syndrome

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
NCF1	653361	7	15626477	autosomal recessive chronic granulomatous disease
KRIT1	889	7	12404106	Cerebral Cavernous Malformations (vascular malformations characterised by abnormally enlarged capillary cavities)
COL1A2	1278	7	17898012	subtle symptoms like recurrent joint subluxation or hypodontia
SHFM1	7979	7	17230488	severe mental retardation, short stature, microcephaly and deafness
RELN	5649	7	16376115, 11592844	Cognitive disruption and altered hippocampus synaptic function
FOXP2	93986	7	16470794	Speech and language impairment and oromotor dysprax
CAV1	857	7	15816560, 14981899	17beta-estradiol-stimulated mammary tumorigenesis
ST7	7982	7	-	no suppression of tumor growth
BRAF	673	7	-	Cardiofaciocutaneous (CFC) syndrome
SHH	6469	7	10852374	Holoprosencephaly, sacral anomalies, and situs ambiguus
HLXB9	3110	7	14663834, 12116275	Currarino syndrome including a presacral mass, sacral agenesis, and anorectal malformation
GATA4	2626	8	10096597	congenital heart disease
NKX3-1	4824	8	15734999	prostate cancer
FGFR1	2260	8	-	Pfeiffer syndrome, Jackson-Weiss syndrome, Antley-Bixler syndrome, osteoglophonic dysplasia, and autosomal dominant Kallmann syndrome 2
CHD7	55636	8	-	CHARGE syndrome
CSN5	10987	8	15735686	TRC8 hereditary kidney cancer

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
EYA1	2138	8	-	branchiootorenal dysplasia syndrome, branchiootic syndrome, and sporadic cases of congenital cataracts and ocular anterior segment anomalies
TRPS1	7227	8	11285235	dominantly inherited tricho-rhino-phalangeal (TRP) syndromes
DMRT1	1761	9	-	failure of testicular development and feminization in male
DMRT2	10655	9	-	defective testis formation in karyotypic males and impaired ovary function in karyotypic females
MLLT3	4300	9	-	neuromotor developmental delay, cerebellar ataxia, and epilepsy
ARF	1029	9	16199529, 12019208	acute myeloid leukemia
CDKN2B	1030	9	10388473	syndrome of cutaneous malignant melanoma and nervous system tumors
BAG1	573	9	15560850	lung tumorigenesis
PAX5	5079	9	-	pathogenesis of lymphocytic lymphomas
GCNT1	2650	9	16778138	T lymphoma cells resistant to cell death
ROR2	4920	9	17632781	basal cell nevus syndrome (BCNS)
PTCH1	5727	9	11922389, 14500378	Primitive neuroectodermal tumors formation
NR5A1	2516	9	14594453	impaired testicular development, sex reversal, and adrenal failure
LMX1B	4010	9	15774843, 11668639, 9837817	nail-patella syndrome
ENG	2022	9	15718503, 16470589	Hereditary hemorrhagic telangiectasia type 1
TSC1	7248	9	14633685	transitional cell carcinoma of the bladder

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
COL5A1	1289	9	16431952, 11391664, 10777716	Structural abnormalities of the cornea and lid
NOTCH1	4851	9	16601454	aortic valve disease (cardiac malformation and aortic valve calcification)
EHMT1	79813	9	16826528, 15805155	9q34 subtelomeric deletion syndrome
KLF6	1316	10	17297474	cellular growth dysregulation and tumorigenesis
GATA3	2625	10	17046739, 16817354, 15994092, 15705923, 11577985, 11389161	HDR (hypoparathyroidism, deafness and renal dysplasia) syndrome
ANX7	310	10	14608035	tumorigenesis
PTEN	5728	10	16938570, 16793127, 16738322, 16288012, 16027169, 15466193, 15001465, 12569555, 12461751, 11553783, 9697695, 12011252	prostate cancer high-grade prostatic intra-epithelial neoplasias
PAX2	5076	10	14569086	renal-coloboma syndrome
FGF8	2253	10	17448458	several human craniofacial disorders
BUB3	9184	10	16600919	short life span that is associated with the early onset of aging-related features
CDKN1C	1028	11	10424812	Beckwith-Wiedemann syndrome
NUP98	4928	11	-	destruction of securin in mitosis
PAX6	5080	11	16866875, 16719277, 16717455, 15480875, 15057935, 12782766, 12552561, 11920832, 11431688, 16646034	eye diseases
WT1	7490	11	8827067, 17931563	congenital genitourinary (GU) anomalies and/or bilateral disease and tumorigenesis
EXT2	2132	11	11137991	type II form of multiple exostoses
ALX4	60529	11	15057119, 9636085	Tibial aplasia, lower extremity mirror image polydactyly,

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
				brachyphalangy, craniofacial dysmorphism and genital hypoplasia
FEN1	2237	11	16978612	neuromuscular and neurodegenerative diseases
SF1	7536	11	17940071, 17200175	mild gonadal dysgenesis and impaired androgenization
FGF3	2248	11	17656375	otodental syndrome
FZD4	8322	11	17103440	complex chromosome rearrangement with multiple abnormalities including growth retardation, facial anomalies, exudative vitreoretinopathy (EVR), cleft palate, and minor digital anomalies
ATM	472	11	10571946, 10363981	High incidence of cancer
H2AX	3014	11	12914700	genomic instability, early onset of various tumors
FLI1	2313	11	15525489	Paris-Trousseau thrombopenia
NFRKB	4798	11	11920839	cellular immunodeficiency, pancytopenia, malformations
PHB2	11331	12	-	enhanced estrogen receptor function
ETV6	2120	12	16643428	a paediatric pre-B acute lymphoblastic leukaemia
CDKN1B	1027	12	16951165, 11042700, 10935480	ErbB2-induced mammary tumor growth
COL2A1	1280	12	10819645	Stickler syndrome
KRT5	3852	12	-	epidermolysis bullosa simplex
MYF6	4618	12	11053684	myopathy and severe course of Becker muscular dystrophy
IGF1	3479	12	15769976	subtle inhibition of intrauterine and postnatal growth
SERCA2	488	12	17116488, 16204033, 11389134	colon and lung cancer
TBX5	6910	12	15289437, 12789647, 12736217, 11572777	maturation failure of conduction system morphology and function in Holt-Oram syndrome

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
TBX3	6926	12	17265068, 16896345, 12668170, 12376101, 12116211	ulnar-mammary syndrome
HNF1A	6927	12	14633861, 12530534	reduced serum apolipoprotein M levels
BRCA2	675	13	15172125	predisposed to breast, ovarian, pancreatic and other cancers
FKHR	2308	13	15489287	Alveolar rhabdomyosarcomas
RB1	5925	13	12531801	Metaphase cytogenetic abnormalities
ZIC2	7546	13	11699604, 11285244	neurological disorderss, behavioral abnormalities
LIG4	3981	13	-	LIG4 syndrome, nonlymphoid tumorigenesis
COCH	1690	14	16078052	unknown
NPAS3	64067	14	12746393	schizophrenia
NKX2-1	7080	14	-	Choreoathetosis, hypothyroidism, pulmonary alterations, neurologic phenotype and secondary hyperthyrotropinemia, and diseases due to transcription factor defects
PAX9	5083	14	16479262, 16333316, 11941488, 11781684	posterior tooth agenesis
BMP4	652	14	16835935	a contiguous gene syndrome comprising anophthalmia, pituitary hypoplasia, and ear anomalies
GCH1	2643	14	-	malignant hyperphenylalaninemia and dopa-responsive dystonia
SIX6	4990	14	10512683	bilateral anophthalmia and pituitary anomalies
RAD51B	5890	14	16778173	centrosome fragmentation and aneuploidy
BCL11B	64919	14	17306224	suppression of lymphomagenesis and thymocyte development
SPRED1	161742	15	-	neurofibromatosis type 1-like syndrome

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BUBR1	701	15	14744753	enhanced tumor development
DLL4	54567	15	-	embryonic lethality due to major defects in arterial and vascular development
FBN1	2200	15	-	Marfan syndrome, isolated ectopia lentis, autosomal dominant Weill-Marchesani syndrome, MASS syndrome, and Shprintzen-Goldberg craniosynostosis syndrome
ALDH1A2	8854	15	-	facilitate posterior organ development and prevent spina bifida
TPM1	7168	15	-	type 3 familial hypertrophic cardiomyopathy
P450SCC	1583	15	11502818	46,XY sex reversal and adrenal insufficiency
BLM	641	15	12242442	the autosomal recessive disorder Bloom syndrome
COUP-TFII	7026	15	15384084	several malformations, pre- and postnatal growth retardation and developmental
SOX8	30812	16	-	the mental retardation found in ATR-16 syndrome
TSC2	7249	16	16027168, 12100629	differential cancer susceptibility
PKD1	5310	16	-	autosomal dominant polycystic kidney disease
CBP	1387	16	11962765	Rubinstein-Taybi syndrome
SOCS1	8651	16	15197228	severe liver fibrosis and hepatitis-induced carcinogenesis
PRM2	5620	16	-	infertility
PRM1	5619	16	-	infertility
ABCC6	368	16	-	pseudoxanthoma elasticum
ERAF	51327	16	-	subtle erythroid phenotype
SALL1	6299	16	16429401	Townes-Brocks syndrome
CBFB	865	16	17022082	delayed cranial ossification, cleft palate, congenital heart anomalies, and feeding difficulties

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
CTCF	10664	16	17962299, 15761865	loss of imprinting of insulin-like growth factor-II in Wilms tumor
WWOX	51741	16	17575124	initiation of tumor development
FOXF1	2294	16	11943666	defects in formation and branching of primary lung buds
FOXC2	2303	16	16910099, 16081467, 15624441, 12719382, 11694548, 11078474	the lymphatic/ocular disorder Lymphedema-Distichiasis
YWHAE	7531	17	-	pathogenesis of small cell lung cancer
HIC1	3090	17	16724116	Miller-Dieker syndrome
LIS1	5048	17	17148952, 16642511, 9760204	abnormal cell proliferation, migration and differentiation in the adult dentate gyrus
P53	7157	17	15583690, 12517413, 12467136, 11695559, 11532857, 11319275	male oral squamous cell carcinomas
PMP22	5376	17	15955700	hereditary neuropathy with liability to pressure palsies
COPS3	8533	17	10851253	Circadian rhythm abnormalities of melatonin in Smith-Magenis syndrome
RAI1	10743	17	17041942, 17024248, 16845274, 15690371, 15565467	Smith-Magenis syndrome
TOP3A	7156	17	-	Smith-Magenis syndrome
SHMT1	6470	17	-	Smith-Magenis syndrome
RNF135	84282	17	17632510	phenotypic abnormalities including overgrowth
NF1	4763	17	16893911, 16835260, 15804420, 15676286, 15103551, 12124168, 9187663, 17103458	neurofibromatosis type 1
SUZ12	23512	17	-	mental impairment in constitutional NF1 microdeletions
MEL-18	7703	17	12196719	breast carcinogenesis
KLHL10	317719	17	-	disrupted spermiogenesis

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
STAT5B	6777	17	15870688	striking amelioration of IL-7-induced mortality and disease development
STAT5A	6776	17	15870688	striking amelioration of IL-7-induced mortality and disease development
BECN1	8678	17	-	autophagy function, and tumor suppressor function
BRCA1	672	17	17420720, 17404506, 15289302	shortened life span and ovarian tumorigenesis
PGRN	2896	17	17168647, 16862115	neurodegeneration
MAPT	4137	17	-	neuronal cell death, neurodegenerative disorders such as Alzheimer's disease, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy
CSH1	1442	17	14642004	Silver-Russell syndrome
POLG2	11232	17	-	mtDNA deletions causes COX deficiency in muscle fibers and results in the clinical phenotype
PRKAR1A	5573	17	15371594	Carney complex, a familial multiple neoplasia syndrome
SOX9	6662	17	17142326, 11606049, 8894698, 8001137	skeletal dysplasias
NHERF1	9368	17	17078868	breast tumours
FSCN2	25794	17	16043865	photoreceptor degeneration, autosomal dominant retinitis pigmentosa
DSG1	1828	18	17194569	diseases of epidermal integrity
DSG2	1829	18	-	ARRHYTHMOGENIC RIGHT VENTRICULAR DYSPLASIA
TCF4	6925	18	17478476	Pitt-Hopkins syndrome, a syndromic mental disorder
FECH	2235	18	10068685	protoporphyria
MC4R	4160	18	12851322, 12639913, 10598807	increased adiposity and linear growth

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
GALR1	2587	18	-	uncontrolled proliferation and neoplastic transformation
SALL3	27164	18	-	18q deletion syndrome
LKB1	6794	19	12218179	Peutz-Jeghers syndrome
PNPLA6	10908	19	15094302	organophosphorus-induced hyperactivity and toxicity
RYR1	6261	19	-	malignant hyperthermia susceptibility, central core disease, and minicore myopathy with external ophthalmoplegia
TGFB1	7040	19	17114585	Aggressive pancreatic ductal adenocarcinoma
RPS19	6223	19	-	Diamond-Blackfan anemia
DMPK	1760	19	10021468	cardiac disease in myotonic dystrophy
CRX	1406	19	10892846	photoreceptor degeneration, Leber congenital amaurosis type III and the autosomal dominant cone-rod dystrophy 2
PRPF31	26121	19	-	retinitis pigmentosa with reduced penetrance
JAG1	182	20	11861489, 11139239, 10590916, 17786115, 11152664, 10534349	Alagille syndrome
PAX1	5075	20	12774041	Klippel-Feil syndrome
GDF5	8200	20	16532400, 12357473	Multiple-synostosis syndrome
HNF4A	3172	20	10905494	monogenic autosomal dominant non-insulin-dependent diabetes mellitus type I
SALL4	57167	20	16790473	Okihiro syndrome
MC3R	4159	20	-	susceptibility to obesity
RAE1	8480	20	16355229	premature separation of sister chromatids, severe aneuploidy and untimely degradation of securin
GNAS	2778	20	17652219, 15579796	reduced activation of a downstream target in epithelial tissues
EDN3	1908	20	-	Hirschsprung disease

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
KCNQ2	3785	20	12700166	epilepsy susceptibility
SOX18	54345	20	17290276	mental retardation
SLC5A3	6526	21	-	brain inositol deficiency
RUNX1	861	21	17394134, 16364766, 15339695, 15061191, 11830488, 11721958, 15297309, 14556655, 11756147, 10684580	The 8p11 myeloproliferative syndrome
DYRK1A	1859	21	12192061	neurological defects, developmental delay
COL6A1	1291	21	-	autosomal dominant disorder, Bethlem myopathy
PRODH	5625	22	17028864	22q11 Deletion syndrome
DGCR2	9993	22	-	DiGeorge syndrome
HIRA	7290	22	9063745, 8111380	DiGeorge syndrome (cranio-facial, cardiac and thymic malformations)
TBX1	6899	22	16969581, 16684884, 15778864, 12539040, 12351571, 11242049	22q11 deletion syndrome and schizophrenia
COMT	1312	22	16848928	22q11.2 deletion syndrome
RTN4R	65078	22	-	schizophrenia susceptibility (schizoaffective disorders are common features in patients with DiGeorge/velocardiofacial syndrome)
PCQAP	51586	22	11414760	DiGeorge syndrome
LZTR1	8216	22	-	DiGeorge syndrome
INI1	6598	22	16912184	pituitary tumorigenesis
MYH9	4627	22	16162639	hematological abnormalities
SOX10	6663	22	11641219	the etiology of Waardenburg/Hirschsprung disease
FBLN1	2192	22	-	limb malformations
PPARA	5465	22	-	prostate cancer
PROSAP2	85358	22	11431708, 12065602	The terminal 22q13.3 deletion syndrome, characterized by severe expressive-language delay, mild mental retardation, hypotonia, joint laxity,

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
				dolichocephaly, and minor facial dysmorphisms
SHOX	6473	X	17881654, 17726696, 16776105, 16319696, 15356038, 15173321, 15118270, 14981722, 14557470, 14513876, 14513875, 12673642, 12510982, 12439897, 12116254, 12035792, 11889214, 11701728, 11546827, 11523902, 11503163, 11408757, 11134233, 10905666, 10878753, 10842291, 10798359, 10749976, 10599728	congenital form of growth failure, the aetiology of "idiopathic" short stature and the growth deficits and skeletal anomalies in Leri Weill, Langer and Turner syndrome
P2RY8	286530	X	15466006	mentally retarded males
NLGN4X	57502	X	-	autism and Asperger syndrome
TRAPPC2	6399	X	-	spondyloepiphyseal dysplasia tarda
RPS4X	6191	X	-	unknown
CSF2RA	1438	X	8950669	growth deficiency
CHRDL1	91851	X	3196642	topographic retinotectal projection and in the regulation of retinal angiogenesis in response to hypoxia
SF3B4	10262	1	24003905, 27127115,	Nager syndrome, Hepatocellular carcinoma and Rodriguez Acrofacial Dysotosis
CTNND2	1501	5	29127138, 25839933	Intellectual disability, epilepsy
AAGAB	79719	15	26608363, 25771163	Buschke-Fischer-Brauer and punctate palmoplantar keratoderma
ABCD1	215	X	26454440, 29136088	adrenoleukodystrophy
AKT3	10000	1	28969385, 27297869	Developmental disorders and breast cancer
ANKRD11	29123	16	28422132, 27605097	KBG syndrome
ANOS1	3730	X	28780519, 25892360	Kallmann syndrome
AP1S2	8905	X	17617514, 23756445,	Mental retardation

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
AR	367	X	29051026	Kennedy's disease and androgen insensitivity
ARSE	415	X	20301713, 23470839	chondrodysplasia punctata
ARX	170302	X	25044608	cognitive disability and epilepsy
ASXL1	171023	20	27616637	myelodysplastic syndromes and chronic myelomonocytic leukemia
ATP7A	538	X	22992316, 24754450	Menkes disease, X-linked distal spinal muscular atrophy, and occipital horn syndrome
ATP8A2	51761	13	20683487	cerebellar ataxia and cognitive disabilities
ATRX	546	X	20301622	cognitive disabilities as well as alpha-thalassemia (ATRX) syndrome
AUTS2	26053	7	26717414	autism spectrum disorders, intellectual disability, and developmental delay
AVPR2	554	X	27565746, 27117808	Nephrogenic Diabetes Insipidus (NDI)
BAG3	9531	10	28211974	cardiomyopathy
BCL11A	53335	2	28891213	Autism and intellectual development
BCOR	54880	X	26573325	sarcoma of the kidney
BMPR1A	657	10	26383923	Intellectual disability
BRWD3	254065	X	24462886, 17668385	cognitive disabilities and X-linked macrocephaly
BTK	695	X	19039656	agammaglobulinemia
CACNA1C	775	12	28493952, 26204268	Autism
CASK	8573	X	28783747, 24927672	FG syndrome 4, intellectual disability and microcephaly
CDH1	999	16	26182300	breast, colorectal, thyroid, gastric and ovarian cancer
CDKL5	6792	X	27265524, 26701947	infantile spasm syndrome (ISSX), also known as X-linked West syndrome, and Rett syndrome (RTT).
CHD2	1106	15	26677509	Neurodevelopmental disorders

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
CHD8	57680	14	26921529, 25989142, 2673379	Autism
CHM	1121	X	27820636	choroideremia
CHRM3	1131	1	26959877	Schizophrenia
CLCN5	1184	X	27117801, 29058463	Dent disease and renal tubular disorders complicated by nephrolithiasis
CNKSR2	22866	X	22511892	Intellectual disability
CNTN4	152330	3	21308999	autism spectrum disorders
CNTNAP2	26047	7	27439707	neurodevelopmental disorders, including Gilles de la Tourette syndrome, schizophrenia, epilepsy, autism, ADHD and intellectual disability
COL11A1	1301	1	21035103	Fibrochondrogenesis, Stickler syndrome and with Marshall syndrome
COL1A1	1277	17	28102596	imperfecta types I-IV, Ehlers-Danlos syndrome type VIIA, Ehlers-Danlos syndrome Classical type, Caffey Disease and idiopathic osteoporosis
CREBBP	1387	16	27342041	Rubinstein-Taybi syndrome (RTS) and acute myeloid leukemia
CRYBB2	1415	22	25489230, 25964531,	Cataracts and prostate cancer
CUL4B	8450	X	24898194	Intellectual disability
CYBB	1536	X	27917630	chronic granulomatous disease (CGD
DCX	1641	X	25868952	pilepsy, cognitive disability, subcortical band heterotopia and lissencephaly syndrome
DICER1	23405	14	24761742	familial tumor susceptibility syndrome
DKC1	1736	X	27570172, 25499969	X-linked dyskeratosis congenita
DLG3	1741	X	19795139	cognitive disability
DMD	1756	X	28247318	uchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), and cardiomyopathy

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
DSC2	1824	18	26310507	arrhythmogenic right ventricular dysplasia-11, and cancer
EBP	10682	X	22121851	Chondrodysplasia punctata 2
EDNRB	1910	13	8852658	Hirschsprung disease type 2
EDA	1896	X	25846883	X-linked hypohidrotic ectodermal dysplasia
EFNB1	1947	X	15959873	craniofrontonasal syndrome
EFTUD2	9343	17	26507355	mandibulofacial dysostosis with microcephaly
EMX2	2018	10	8528262	schizencephaly
EP300	2033	22	25712426	Rubinstein-Taybi syndrome and epithelial cancer
ERF	2077	19	26097063	craniosynostosis
ERMARD	55780	6	24056535	Periventricular nodular heterotopia
EXT1	2131	8	24009674	Multiple osteochondromas
EYA4	2070	6	15735644	Cardiomyopathy and hearing loss
F8	2157	X	28777843	hemophilia A
F9	2158	X	28007939	hemophilia B or Christmas disease
FAM58A	92002	X	18297069	STAR syndrome
FANCB	2187	X	21910217	VACTERL syndrome
FAS	355	10	21490157	Autoimmune lymphoproliferative syndrome
FGD1	2245	X	27199457	dysplasia in Aarskog-Scott syndrome and a syndromatic form of X-linked cognitive disability
FLCN	201163	17	28970150	Birt-Hogg-Dube syndrome
FLG	2312	1	21514438	ichthyosis vulgaris
FLNA	2316	X	22238415	Periventricular nodular heterotopias, otopalatodigital syndromes, frontometaphyseal dysplasia, Melnick-Needles syndrome, and X-linked

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
				congenital idiopathic intestinal pseudoobstruction
FOXG1	2290	14	28851325	Rett syndrome
FRMD7	90167	X	25678693	congenital nystagmus
FTSJ1	24140	X	18401546	cognitive disability
GATA2	2624	3	21670465, 21892158	monocytopenia and mycobacterial infection syndrome and Emberger syndrome
GATA6	2627	18	25706805	congenital defects and cardiomyopathy
GDI1	2664	X	21736009	cognitive disability
GJA5	2702	1	25992486	atrial fibrillation
GJA8	2703	1	28526010	zonular pulverulent cataracts, nuclear progressive cataracts, and cataract-microcornea syndrome
GK	2710	X	10851254	glycerol kinase deficiency
GLA	2717	X	28723748	Fabry disease
GLI2	2736	2	25974718	Greig cephalopolysyndactyly syndrome, Pallister-Hall syndrome, preaxial polydactyly type IV, postaxial polydactyly types A1 and B
GLMN	11146	1	15689436	glomuvenous malformations
GPC3	2719	X	28371070	Simpson-Golabi-Behmel syndrome
GRIA3	2892	X	19449417	Intellectual disability
GRIN2A	2903	16	27683935	epilepsy and speech disorder
GRIN2B	2904	12	27818011	neurodevelopmental disorders autism, attention deficit hyperactivity disorder, epilepsy and schizophrenia
HCCS	3052	X	-	microphthalmia syndrome
HDAC4	9759	2	20691407	Mental retardation
HMGA2	8091	12	25809938	Silver-Russell syndrome
HNF1B	6928	17	27838256	Intellectual disability
HNRNPK	3190	9	26173930	Intellectual disability
HPRT1	3251	X	29185864	Lesch-Nyhan syndrome or gout

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
HNRNPU	3192	1	28393272	epileptic encephalopathy and intellectual disability
IDS	3423	X	27246110	Hunter syndrome
IGF1R	3480	15	21811077	Familial short statute
IKBKG	8517	X	27037530	inncontinentia pigmenti, hypohidrotic ectodermal dysplasia, and immunodeficiencies
IL1RAPL1	11141	X	21933724	intellectual disability
KANSL1	284058	17	20301783	intellectual disability
KAT6B	23522	10	26334766	Say-Barber-Biesecker/Young-Simpson syndrome
KCNH2	3757	7	24530480	long QT syndrome type 2
KDM5C	8242	X	25666439	cognitive disability
KDM6A	7403	X	23076834	Kabuki syndrome
KIAA2022	340533	X	27358180	cognitive disability and epilepsy
KIF11	3832	10	22653704	microcephaly
KMT2A	4297	11	28911906	Acute lymphoid leukemias and acute myeloid leukemias
KMT2D	8085	12	27530205	Kabuki syndrome
L1CAM	3897	X		Masa syndrome and L1 syndrome
LAMP2	3920	X	28627787	Danon disease
LDLR	3949	19	28873201	Familial hypercholesterolemia
LEMD3	23592	12	26694706	Buschke-Ollendorff syndrome and melorheostosis
LHX4	89884	1	25871839	hypopituitarism
LMNA	4000	1	20127487	cardiomyopathy
LRP5	4041	11	27228167	familial exudative vitreoretinopathy
MAGEL2	54551	15	26365340,	Prader-Willi syndrome (PWS)
MAGT1	84061	X	24130152	intellectual disability
MAOA	4128	X	8211186	Mental retardation
MAP2K2	5605	19	25487361	cardiofaciocutaneous syndrome
MBD5	55777		27786435, 25271084, 24885232	Microcephaly, intellectual disabilities, speech impairment, and seizures

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
MECP2	4204	X	29141583	Rett syndrome
MED13L	23389	12	28371282, 28645799	Intellectual disability
MEF2C	4208	5	27255693	cognitive disability, epilepsy, and cerebral malformation
MEIS2	4212	15	25712757	Intellectual disability
MEN1	4221	11	9510467, 15105049, 21763627	Multiple Endocrine Neoplasia type 1
MID1	4281	X	25304119	Opitz syndrome
MLH1	4292	3	15942939	colon cancer
MNX1	3110	7	24095820	Currarino syndrome
MSH2	4436	2	26498247	hereditary nonpolyposis colon cancer
MSH6	2956	2	6099011	hereditary nonpolyposis colon cancer, colorectal cancer, and endometrial cancer
MTAP	4507	9	22464254	diaphyseal medullary stenosis with malignant fibrous histiocytoma (DMSMFH).
MTM1	4534	X	21488203	X-linked myotubular myopathy
MYBPC3	4607	11	27348999	familial hypertrophic cardiomyopathy
MYLK	4638	3	28602422	Megacystis Microcolon Intestinal Hypoperistalsis Syndrome
MYT1L	23040	2	22547139	schizophrenia
NDP	4693	X	27217716	Norrie disease
NF2	4771	22	11159946	neurofibromatosis type II
NFIX	4784	19	26200704	Marshall-Smith syndrome or Sotos-like syndrome
NHS	4810	X	28557584	Nance-Horan syndrome
NIPBL	25836	5	26701315	Cornelia de Lange syndrome
NODAL	4838	10	19064609	Cardiovascular malformations
NOG	9241	17	25391606	symphalangism (SYM1) and multiple synostoses syndrome (SYNS1)
NR0B1	190	X	25968435	congenital adrenal hypoplasia and hypogonadotropic hypogonadism

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
NRXN1	9378	2	26279266	Pitt-Hopkins-like syndrome-2 and schizophrenia
NSDHL	50814	X	26014843	CHILD syndrome
NXF5	55998	X	11566096	Mental retardation
NYX	60506	X	26234941	X-linked congenital stationary night blindness
OCRL	4952	X	27059748	oculocerebrorenal syndrome of Lowe and also Dent disease
OFD1	8481	X	28371265	oral-facial-digital syndrome type I and Simpson-Golabi-Behmel syndrome type 2
OPHN1	4983	X	17845870	X-linked cognitive disability
OTC	5009	X	26446336	Hyperammonemia
OTX2	5015	14	27299576, 28388256	syndromic microphthalmia 5 and pituitary hormone deficiency 6
PAFAH1B1	5048	17	11754098	Lissencephaly
PAK2	5062	3	21841781	intellectual disability
PAK3	5063	X	18523455	intellectual disability
PCDH19	57526	X	27179713	epileptic encephalopathy and autism
PDHA1	5160	X	10679936	X-linked Leigh syndrome
PGK1	5230	X	16567715	neurological impairment
PHEX	5251	X	27840894	Hypophospatemic rickets
PHF6	84295	X	22190899	cognitive disability and epilepsy
PHF8	23133	X	17594395	Mental retardation and cleft palate
PIGA	5277	X	24706016	encephalopathies
PITX3	5309	10	16565358	Ocular and neurological disorders
PKP2	5318	12	27030002	cardiomyopathy
PLP1	5354	X	27793435	Pelizaeus-Merzbacher disease and spastic paraplegia type 2
POLR1D	51082	13	24603435	Treacher Collins syndrome (TCS)
PORCN	64840	X	23696273	focal dermal hypoplasia
PQBP1	10084	X	21204222	cognitive disability

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
PRPS1	5631	X	26089585	Charcot-Marie-Tooth disease and Arts syndrome
PRRT2	112476	16	22744660	paroxysmal kinesigenic dyskinesias
PTHLH	5744	12	26733284	osteochondoplasia
PTPN11	5781	12	28328117	Noonan syndrome
RAB39B	116442	X	20159109	cognitive disability, epilepsy, and macrocephaly
RASA1	5921	5	26969842	capillary malformations and Parkes Weber syndrome
RBFOX1	54715	16	26174448	Epilepsy
RET	5979	10	-	Hirschsprung disease
RP2	6102	X	16969763	Retinal dystrophies
RPS17	6218	15	23812780	Diamond-Blackfan anemia
RPS24	6229	10	17186470	Diamond-Blackfan anemia
RPS26	6231	12	22045982	Diamond-Blackfan anemia
RPS6KA3	6197	X	26297997	Coffin-Lowry syndrome
RS1	6247	X	26043410	retinoschisis
SCN2A	6326	2	26291284	Epilepsy and autism
SCN5A	6331	3	28069705	Long QT syndrome type 3
SDHAF2	54949	11	21224366	paraganglioma
SDHB	6390	1	27839933	paraganglioma
SDHC	6391	1	26652933	paraganglioma
SDHD	6392	11	28924001	paraganglioma
SETBP1	26040	18	28346496	Schinzel-Giedion syndrome
SETD5	55209	3	27375234	Intellectual disability
SGCE	8910	7	26783545	Myoclonus dystonia
SH2B1	25970	16	23160192	Maladaptive behaviors and obesity
SH2D1A	4068	X	-	Lymphoproliferative syndrome
SIX3	6496	2	19346217	holoprosencephaly
SLC16A12	387700	10	18304496	Juvenile cataracts and renal glucosuria
SLC16A2	6567	X	27805744	Allan-Herndon-Dudley syndrome
SLC2A1	6513	1	25532859	Paroxysmal exertion-induced dyskinesia

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
SLC4A10	57282	2	18413482	Epilepsy and mental retardation
SLC6A8	6535	X	24137762	Mental retardation
SLC9A6	10479	X	25044251	cognitive disability
SMAD3	4088	15	22803640	Cardiovascular malformations and aneurysms
SMAD4	4089	18	18823382	pancreatic cancer, juvenile polyposis syndrome, and hereditary hemorrhagic telangiectasia syndrome
SMARCA4	6597	19	23775540	Rhabdoid tumor predisposition syndrome
SMARCB1	6598	22	28338502	Rhabdoid tumor predisposition syndrome
SMS	6611	X	23696453	intellectual disability
SNURF	8926	15	22511895	Prader-Willi Syndrome
SOX11	6664	2	6543203	Autism and mental retardation
SOX5	6660	12	23498568	Mental retardation
SPINK1	6690	5	27159572	hereditary pancreatitis and tropical calcific pancreatitis
SRY	6736	Y	7987333	gonadal dysgenesis
STK11	6794	19	29141581	Peutz-Jeghers syndrome and cancer
STS	412	X	26421812	X-linked ichthyosis (XLI)
STXBP1	6812	9	26865513	infantile epileptic encephalopathy-4
SYN1	6853		22807112	neuronal degeneration such as Rett syndrome
SYNGAP1	8831	6	23161826	intellectual disability and autism
TAB2	23118	6	25940952	congenital heart defects
TBX20	57057	7	26118961	cardiac pathologies
TBX22	50945	X	22851992	Cleft palate
TBX4	9496	17	15106123	Small patella syndrome
TCF12	6938	15	26068201	Anaplastic oligodendroglioma
TDGF1	6997	3	12073012	forebrain defects
TFAP2B	7021	6	24507797	Char syndrome
TGFBR1	7046	9	21358634	Ferguson-Smith disease (FSD)

Gene Symbol	Entrez Gene ID	Chromosome	PubMed (PMID)	Disorder/ Syndrome
TGFBR2	7048	3	28344185	Syndrome, Loeys-Deitz Aortic Aneurysm Syndrome
TGIF1	7050	18	16962354	holoprosencephaly type 4
TIMM8A	1678	X	20301395	Jensen syndrome
TNNI3	7137	19	18006163	cardiomyopathy
TP63	8626	3	11462173	ectodermal dysplasia, cleft lip/palate, and split-hand/foot malformation
TSPAN7	7102	X	19339915	cognitive disability and neuropsychiatric diseases
UBE2A	7319	X	16909393	cognitive disability
UBE3A	7337	15	28559284	autism
UPF3B	65109	X	22609145	Mental retardation
VEGFA	7422	6	20420808	Cardiovascular defects
WDR45	11152	X	27030146	neurodegeneration
XIAP	331	X	26182687	dysgammaglobulinemia
YAP1	10413	11	24462371	hearing loss, intellectual disability, hematuria, and orofacial clefting
ZC4H2	55906	X	23623388	cognitive disability
ZDHHC9	51114	X	28687527	cognitive disability
ZEB2	9839	2	15121779	Mowat-Wilson syndromw
ZFPM2	23414		24769157	Cardiovascular malformations
ZIC1	7545	3	24782033	Hepatocellular carcinoma
ZIC3	7547	X	24123890	X-linked visceral heterotaxy
ZIC4	84107	3	21204220	Danny-Walker malformation
ZNF41	7592	X	14628291	cognitive disability
ZNF674	641339	X	22126752	cognitive disability
ZNF711	7552	X	21384559	cognitive disability
CACNA1A	773	19		Neurological disorders

Compositions

Episomal Vectors

[0095] Described herein are compositions useful as components for targeting transcriptional activation domains to genetic control elements to increase transcription of an endogenous gene and thereby treat a disease or condition associated with, exacerbated by, or caused by reduced transcription of a gene, reduced amount of a gene product, or reduced activity of a gene product.

The components include guide RNAs, scaffold RNAs, scaffold RNA ligands, CRISPR nucleases, transcriptional activation domains, affinity tag(s), affinity tag ligand(s), fusion proteins of one or more thereof, and combinations thereof. The components also include episomal vectors that encode one or more guide RNAs, scaffold RNAs, scaffold RNA ligands, CRISPR nucleases, transcriptional activation domains, affinity tag(s), affinity tag ligand(s), fusion proteins of one or more thereof, and combinations thereof. The episomal vectors can be single- or double-stranded DNA, single-stranded RNA, or double-stranded RNA.

[0096] In one embodiment, an episomal vector encoding a CRISPR nuclease, such as a catalytically inactive CRISPR nuclease is be provided. In some cases, the episomal vector encodes a CRISPR nuclease fused to one or more transcriptional activation domains. In some cases, the episomal vector encodes a CRISPR nuclease fused to one or more affinity tags. In some cases, the episomal vector encodes a CRISPR nuclease fused to one or more affinity tags and one or more transcriptional activation domains. CRISPR nuclease fusion proteins can contain transcriptional activator domain(s) and/or affinity tag(s) fused at the amino-terminus of the CRISPR nuclease, at the carboxy terminus, or a combination thereof. Additionally or alternatively, the CRISPR nuclease can be modified by the insertion of transcriptional activator domain(s) and/or affinity tag(s) within a surface loop. The episomal vector (*e.g.*, AAV vector) can contain a promoter that is operably linked to the CRISPR nuclease or CRISPR nuclease fusion protein. The promoter can be a promoter that is endogenous to a viral source from which the episomal vector is derived. For example, where the episomal vector is an AAV vector, the promoter can be an endogenous AAV promoter. Alternatively, the promoter can be a promoter that is heterologous to the viral source form which the episomal vector is derived. For example, where the episomal vector is an AAV vector, the promoter can be a non-AAV promoter. The promoter can be a promoter of a gene targeted for transcriptional activation (*e.g.*, a gene selected from Table 1) or a promoter that is heterologous to the targeted gene. The promoter can be constitutive (*e.g.*, a CMV promoter, CAG promoter, CBA promoter, EF1a promoter, PGK promoter, *etc.*), tissue specific (*e.g.*, a synapsin, camKIIa, GFAP, RPE, ALB, TBG, MBP, MCK, TNT, or aMHC, promoter, and the like), or inducible (*e.g.*, tetracycline inducible).

[0097] In one embodiment, an episomal vector encoding a zinc finger nuclease is provided. In some cases, the episomal vector encodes a zinc finger nuclease fused to one or more

transcriptional activation domains. In some cases, the episomal vector encodes a zinc finger nuclease fused to one or more affinity tags. In some cases, the episomal vector encodes a zinc finger nuclease fused to one or more affinity tags and one or more transcriptional activation domains. Zinc finger nuclease fusion proteins can contain transcriptional activator domain(s) and/or affinity tag(s) fused at the amino-terminus of the zinc finger nuclease, at the carboxy terminus, or a combination thereof. The episomal vector (*e.g.*, AAV vector) can contain a promoter that is operably linked to the zinc finger nuclease or zinc finger nuclease fusion protein. The promoter can be a promoter that is endogenous to a viral source from which the episomal vector is derived. For example, where the episomal vector is an AAV vector, the promoter can be an endogenous AAV promoter. Alternatively, the promoter can be a promoter that is heterologous to the viral source form which the episomal vector is derived. For example, where the episomal vector is an AAV vector, the promoter can be a non-AAV promoter. The promoter can be a promoter of a gene targeted for transcriptional activation (*e.g.*, a gene selected from Table 1) or a promoter that is heterologous to the targeted gene. The promoter can be constitutive (*e.g.*, a CMV promoter, CAG promoter, CBA promoter, EF1a promoter, PGK promoter, *etc.*), tissue specific (*e.g.*, a synapsin, camKIIa, GFAP, RPE, ALB, TBG, MBP, MCK, TNT, or aMHC, promoter, and the like), or inducible (*e.g.*, tetracycline inducible).

[0098] In one embodiment, an episomal vector encoding a TALEN is provided. In some cases, the episomal vector encodes a TALEN fused to one or more transcriptional activation domains. In some cases, the episomal vector encodes a TALEN fused to one or more affinity tags. In some cases, the episomal vector encodes a TALEN fused to one or more affinity tags and one or more transcriptional activation domains. TALENs can contain transcriptional activator domain(s) and/or affinity tag(s) fused at the amino-terminus, at the carboxy terminus, or a combination thereof. The episomal vector (*e.g.*, AAV vector) can contain a promoter that is operably linked to the TALEN. The promoter can be a promoter that is endogenous to a viral source from which the episomal vector is derived. For example, where the episomal vector is an AAV vector, the promoter can be an endogenous AAV promoter. Alternatively, the promoter can be a promoter that is heterologous to the viral source form which the episomal vector is derived. For example, where the episomal vector is an AAV vector, the promoter can be a non-AAV promoter. The promoter can be a promoter of a gene targeted for transcriptional activation (*e.g.*, a gene selected from Table 1) or a promoter that is heterologous to the targeted gene. The

promoter can be constitutive (*e.g.*, a CMV promoter, CAG promoter, CBA promoter, EF1a promoter, PGK promoter, *etc.*), tissue specific (*e.g.*, a synapsin, camKIIa, GFAP, RPE, ALB, TBG, MBP, MCK, TNT, or aMHC, promoter, and the like), or inducible (*e.g.*, tetracycline inducible).

[0099] In one embodiment, an episomal vector encoding a guide RNA is provided. The guide RNA can be a small guide RNA. The guide RNA can be a component of a synergistic activation mediator (*e.g.*, as described in Zhang *et al.*, Scientific Reports 5, Article No. 16277 (2015); and Konermann *et al.*, 2015, Nature 517:583-8). The episomal vector (*e.g.*, AAV vector) can contain a promoter that is operably linked to the guide RNA. The promoter can be a promoter that is endogenous to a viral source from which the episomal vector is derived. For example, where the episomal vector is an AAV vector, the promoter can be an endogenous AAV promoter. Alternatively, the promoter can be a promoter that is heterologous to the viral source form which the episomal vector is derived. For example, where the episomal vector is an AAV vector, the promoter can be a non-AAV promoter. The promoter can be a promoter of a gene targeted for transcriptional activation (*e.g.*, a gene selected from Table 1) or a promoter that is heterologous to the targeted gene. The promoter can be constitutive (*e.g.*, a CMV promoter, CAG promoter, CBA promoter, EF1a promoter, PGK promoter, *etc.*), tissue specific (*e.g.*, a synapsin, camKIIa, GFAP, RPE, ALB, TBG, MBP, MCK, TNT, or aMHC, promoter, and the like), or inducible (*e.g.*, tetracycline inducible).

[0100] In some embodiments, the episomal vector encodes both a CRISPR nuclease and a guide RNA. In some cases, the CRISPR nuclease is operably linked to a promoter and the guide RNA is operably linked to a different promoter. In some cases, the two promoters are the same. In some cases, the two promoters are different. In some cases, both promoters are inducible. In some cases, both promoters are tissue specific. In some cases, both promoters are constitutive. In some cases, one promoter is constitutive and the other promoter is tissue specific. In some cases, one promoter is constitutive and the other promoter is inducible. In some cases, one promoter is tissue specific and the other is inducible.

[0101] In some embodiments, the episomal vector encodes a scaffold RNA, such as a scaffold RNA described in WO 2016/054106. In some embodiments, the episomal vector also encodes a CRISPR nuclease. Additionally or alternatively, the episomal vector can also encode one or

more transcriptional activation domain(s). In some cases, the transcriptional activation domain(s) are fused to a binding element that binds to the scaffold RNA (*e.g.*, binds to an ms2,f6, PP7, com, or L7a sequence of a scaffold RNA).

[0102] In some embodiments, two or more different episomal vector are provided. For example, an episomal vector encoding a CRISPR nuclease and a separate episomal vector encoding a guide RNA can be provided. Alternatively, an episomal vector encoding a CRISPR nuclease and a guide RNA can be provided and a separate episomal vector encoding one or more transcriptional activation domain(s) can be provided. In some cases, the one or more transcriptional activation domains are fused to a binding element that binds a scaffold RNA (*e.g.*, binds a guide RNA of an SAM). In some cases, the one or more transcriptional activation domains are fused to a binding element that binds an affinity tag of a CRISPR nuclease. In some embodiments, an episomal vector encoding a scaffold RNA is provided and a separate episomal vector is provided that encodes one or more transcriptional activation domain(s) fused to a binding element that binds the scaffold RNA.

[0103] In some embodiments, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a gene listed in Table 1, or a gene in the same pathway or a parallel pathway as a gene listed in Table 1. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of a gene listed in Table 1, or a gene in the same pathway or a parallel pathway as a gene listed in Table 1.

[0104] In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) to *SIM1*, *Leptin*, *Leptin receptor*, *MC4R*, *SCN2A*, *SETD5*, *PAX6*, *PKD1*, *MC3R*, *POMC*, *STAT3*, *STAT5*, *SOCS3*, *GHR*, *NPY*, *NPY1R*, *NPY2R*, *NPY5R*, *PYY*, *AMPK (PRKAA1*, *PRKAA2*, *PRKAB1*, *PRKAB2*, *PRKAG1*, *PRKAG2*, *PRKAG3)*, *OXT*, *JAK2*, *SHP2*, *NOS3*, *NROB2*, *BRS3*, *CARTPT*, *FABP4*, *HTR2C*, *IL6*, *NHLH2*, *NMU*, *NPB*, *NPBWRI*, *PNPLA2*, *UCP3*, *ADIPOQ*, *APOA5*, *ARNT2*, *ASIP*, *C1QTNF2*, *C3AR1*, *CCK*, *CPT1B*, *CSF2*, *DGAT1*, *DGAT2*, *GHRL*, *GHSR*, *HSD11B1*, *HTR7*, *INSIG1*, *INSIG2*, *LIPC*, *NMURI*, *NMUR2*, *NPBWR2*, *NTS*, *PPARGC1A*, *PPY*, *RETN*, *SIRT1*, *TGFBR2*, *WDTC1*, *or FOXO1*.

[0105] In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *SIM1*, *Leptin*, *Leptin receptor*, *MC4R*, *SCN2A*, *SETD5*, *PAX6*, *PKD1*, *MC3R*, *POMC*, *STAT3*, *STAT5*, *SOCS3*, *GHR*, *NPY*, *NPY1R*, *NPY2R*, *NPY5R*, *PYY*, *AMPK (PRKAA1*, *PRKAA2*, *PRKAB1*, *PRKAB2*, *PRKAG1*, *PRKAG2*, *PRKAG3)*, *OXT*, *JAK2*, *SHP2*, *NOS3*, *NROB2*, *BRS3*, *CARTPT*, *FABP4*, *HTR2C*, *IL6*, *NHLH2*, *NMU*, *NPB*, *NPBWRI*, *PNPLA2*, *UCP3*, *ADIPOQ*, *APOA5*, *ARNT2*, *ASIP*, *C1QTNF2*, *C3AR1*, *CCK*, *CPT1B*, *CSF2*, *DGAT1*, *DGAT2*, *GHRL*, *GHSR*, *HSD11B1*, *HTR7*, *INSIG1*, *INSIG2*, *LIPC*, *NMURI*, *NMUR2*, *NPBWR2*, *NTS*, *PPARGC1A*, *PPY*, *RETN*, *SIRT1*, *TGFBR2*, *WDTC1*, *or FOXO1*.

[0106] In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *SIM1*. In some cases, the the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *SIM1*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *SIM1*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *MC4R*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *MC4R*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *MC4R*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *PDK1*. In some cases, the the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *PDK1*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *PDK1*. In some cases, the

episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *SETD5*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *SETD5*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *SETD5*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *SCN2A*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *SCN2A*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *SCN2A*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *PAX6*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *PAX6*. In some cases, the episomal vector encodes a zinc finger nuclease or TALEN that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *PAX6*.

[0107] In some embodiments, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a gene listed in Table 1, or a gene in the same pathway or a parallel pathway as a gene listed in Table 1. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of a gene listed in Table 1, or a gene in the same pathway or a parallel pathway as a gene listed in Table 1.

[0108] In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) to *SIM1*, *Leptin*,

Leptin receptor, MC4R, SCN2A, SETD5, PAX6, PKD1, MC3R, POMC, STAT3, STAT5, SOCS3, GHR, NPY, NPY1R, NPY2R, NPY5R, PYY, AMPK (PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1, PRKAG2, PRKAG3), OXT, JAK2, SHP2, NOS3, NROB2, BRS3, CARTPT, FABP4, HTR2C, IL6, NHLH2, NMU, NPB, NPBWRI, PNPLA2, UCP3, ADIPOQ, APOA5, ARNT2, ASIP, C1QTNF2, C3AR1, CCK, CPT1B, CSF2, DGAT1, DGAT2, GHRL, GHSR, HSD11B1, HTR7, INSIG1, INSIG2, LIPC, NMURI, NMUR2, NPBWR2, NTS, PPARGC1A, PPY, RETN, SIRT1, TGFBR2, WDTC1, or FOXO1.

[0109] In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *SIM1*, *Leptin*, *Leptin receptor*, *MC4R*, *SCN2A*, *SETD5*, *PAX6*, *PKD1*, *MC3R*, *POMC*, *STAT3*, *STAT5*, *SOCS3*, *GHR*, *NPY*, *NPY1R*, *NPY2R*, *NPY5R*, *PYY*, *AMPK (PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1, PRKAG2, PRKAG3)*, *OXT*, *JAK2*, *SHP2*, *NOS3*, *NROB2*, *BRS3*, *CARTPT*, *FABP4*, *HTR2C*, *IL6*, *NHLH2*, *NMU*, *NPB*, *NPBWRI*, *PNPLA2*, *UCP3*, *ADIPOQ*, *APOA5*, *ARNT2*, *ASIP*, *C1QTNF2*, *C3AR1*, *CCK*, *CPT1B*, *CSF2*, *DGAT1*, *DGAT2*, *GHRL*, *GHSR*, *HSD11B1*, *HTR7*, *INSIG1*, *INSIG2*, *LIPC*, *NMURI*, *NMUR2*, *NPBWR2*, *NTS*, *PPARGC1A*, *PPY*, *RETN*, *SIRT1*, *TGFBR2*, *WDTC1*, *or FOXO1*.

[0110] In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *SIM1*. In some cases, the the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *SIM1*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *SIM1*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *MC4R*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *MC4R*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *MC4R*. In some cases, the episomal vector encodes a guide or scaffold RNA

that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *PDK1*. In some cases, the the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *PDK1*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *PDK1*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *SETD5*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *SETD5*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *SETD5*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *SCN2A*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *SCN2A*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *SCN2A*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a control region (*e.g.*, promoter region or enhancer region) of *PAX6*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) a promoter region of *PAX6*. In some cases, the episomal vector encodes a guide or scaffold RNA that hybridizes to or specifically hybridizes to (*e.g.*, under stringent hybridization conditions) an enhancer region of *PAX6*.

[0111] In some cases, the targeting region of the guide RNA is encoded by, specifically hybridizes to, or is fully complementary to: SEQ ID NO:1 (GACACGGAATTCATTGCCAG), SEQ ID NO:2 (CTGCGGGTTAGGTCTACCGG), SEQ ID NO:3 (GTTGAGCGCTCAGTCCAGCG), SEQ ID NO:4 (TCCCGACGTCGTGCGCGACC), or SEQ ID NO:5 (GCTCTGAATCTTACTACCCG). In some cases, the targeting region of the guide

RNA is encoded by, specifically hybridizes to, or is fully complementary to: SEQ ID NO:6 (GCTGTTAACTAAAGACAGGG), SEQ ID NO:7 (GTGGTCTGGGTGATCTCATG), SEQ ID NO:8 (GACAAAGGAACATCTGAGAGG), SEQ ID NO:9 (GTGATCTCATGGGGAAGAGG), or SEQ ID NO:10 (GGCTTTGATCGTGGTCTGGG). In some cases, the targeting region of the guide RNA is encoded by, specifically hybridizes to, or is fully complementary to: SEQ ID NO:11 (GCGAGCCCAGTCGCGTGGGG), SEQ ID NO:12 (GCCAAGAATTGGCCAAAGGG), SEQ ID NO:34 (GTCAAAGGGGCATATGGAAGG), SEQ ID NO:35 (GGGAAGAAAGCCCCACTTGG), SEQ ID NO:36 (GCCCAGTCGCGTGGGGGGGG), or SEQ ID NO:37 (GGAGCGCGAGTGTCACTCGG). In another embodiment, the targeting region of the guide RNA is encoded by, specifically hybridizes to, or is fully complementary to: SEQ ID NO:38 (GCTCACTGTAGGACCCGAGCC), SEQ ID NO:39 (GACGCGGCGCTCATTGGCCAA), SEQ ID NO:40 (CGAGCCGCGAGCCCAGTCGCG), SEQ ID NO:41 (TCCCCCCCCCCCCCCACGCGA), SEQ ID NO:42 (GTCACTCACCCCGATTGGCCA), or SEQ ID NO:43 (CGCGAGCCCAGTCGCGTGGGG). In some embodiments, the targeting region of the guide RNA is encoded by, specifically hybridizes to, or is fully complementary to: SEQ ID NO:44 (GTTGGCTTATCCAAACATCTC), SEQ ID NO:45 (ATGTTAAGCAAGGGTAATAGA), SEQ ID NO:46 (CTGTGAAAGGAATACAATTCA), SEQ ID NO: 47 (GCCAATTCTTGGCAACCGAGC), SEQ ID NO:48 (GAATTGGCCAAAGGGAGGGGT), or SEQ ID NO:49 (AATTAGCAGACAGCTTGGTAC). In some embodiments, the targeting region of the guide RNA is encoded by, specifically hybridizes to, or is fully complementary to: SEQ ID NO:50 (CTGGCTGATTCCCGAGGATTT), SEQ ID NO:51 (CACTGAATACGGATTGGTCAG), SEQ ID NO:52 (GATGTCTCAGAACCACTGAAT), SEQ ID NO:53 (AACCACTGAATACGGATTGGT), or SEQ ID NO:54 (ACCAATCCGTATTCAGTGGTT). In some embodiments, the targeting region of the guide RNA is encoded by, specifically hybridizes to, or is fully complementary to: SEQ ID NO:55 (GGCGCGGGGCGGACGGGGCGA), SEQ ID NO:56 (GCGCCCCGGGAACGCGTGGGG), SEQ ID NO:57 (CGCCCCGCGCCGCGCGGGGAG), SEQ ID NO:58 (TCCGCCCCGCGCCGCGCGGGG), SEQ ID NO:59 (GGAACGCGTGGGGCGGAGCTT), SEQ ID NO:60 (GCCCCGCGCCGCGCGGGGAGG), SEQ ID NO:61

(TGCGCCCCGGGAACGCGTGGG), SEQ ID NO:62 (GAACGCGTGGGGCGGAGCTTC), SEQ ID NO:63 (GCGGCGCGGGGCGGACGGGGC), or SEQ ID NO:64 (CCCGTCCGCCCCGCGCCGCGC). In some embodiments, the targeting region of the guide RNA is encoded by, specifically hybridizes to, or is fully complementary to: SEQ ID NO:65 (GGCCCACTCGCCGCCAATCAG), SEQ ID NO:66 (GGAAGCCGCCGGGGCCGCCTA), SEQ ID NO:67 (TGATTGGCGGCGAGTGGGCCA), SEQ ID NO:68: (GCCGCCAATCAGCGGAAGCCG), SEQ ID NO:69: (GGCGGCTTCCGCTGATTGGCG), SEQ ID NO:70: (CCGCCAATCAGCGGAAGCCGC), SEQ ID NO:71: (AGCCGCCGGGGCCGCCTAGAG), SEQ ID NO:72: (GCTTCCGCTGATTGGCGGCGA), SEQ ID NO:73: (CGGCGAGTGGGCCAATGGGTG), or SEQ ID NO:74: (CCAATGGGTGCGGGGCGGTGG). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:75 (GGCTGCCGGGGCCGCCTAAAG), SEQ ID NO:76 (GGAGGCTGCCGGGGCCGCCTA), SEQ ID NO:77 (GCCGCCAATCAGCGGAGGCTG), SEQ ID NO:78 (CCGCCAATCAGCGGAGGCTGC), SEQ ID NO:79 (TGGCCGGTGCGCCGCCAATCA), SEQ ID NO:80 (GGCCGGTGCGCCGCCAATCAG), SEQ ID NO:81(CGGCGCACCGGCCAATAAGTG), SEQ ID NO:82(ATAAGTGTGGGGCGGTGGGCG), SEQ ID NO:83 (CCAATAAGTGTGGGGCGGTGG), or SEQ ID NO:84 (CAATAAGTGTGGGGCGGTGGG). In some embodiments, the targeting region of the guide RNA is encoded by or specifically hybridizes to: SEQ ID NO:85: CCTTTCTATGACCTAGTCGG, SEQ ID NO:86: CAGAATCAGTAACGCACTGT, SEQ ID NO:87: GAAACCAGGAGAGATAACCC, SEQ ID NO:88: GGACCCCAGATATTCTGGAA, SEQ ID NO:89: TTATTGTTGACTTAACGAAG, SEQ ID NO:90: AAAAAGAAGCAAATAGCTAA, or SEQ ID NO:91: (AGAATCAGTAACGCACTGTA). In some embodiments, the targeting region of the guide RNA is encoded by, specifically hybridizes to, or is fully complementary to: SEQ ID NO:92 (TGTTGGTTTATTGGACCCCAGATATTC), SEQ ID NO:93 (TGTTGGAGAAAATTAACTTAGTGCATA), or SEQ ID NO:94 (TGTTGGTATAACTGCCACTAGAGGGCT). In some embodiments, the targeting region of the guide RNA is encoded by, specifically hybridizes to, or is fully complementary to SEQ ID NO:95 (AGGAGCCGGGACCCACCGG).

[0112] In some cases, the targeting region of the guide RNA is encoded by, specifically hybridizes to, or is fully complementary to a sequence that is orthologous and/or homologous to a region of a mouse or human genome corresponding to, or targeted by an sgRNA comprising, one of SEQ ID NOs:1-12, or 34-95. In some cases, the guide RNA is encoded by, specifically hybridizes to, or is fully complementary to a sequence that is 90%, 95%, or 99% identical to, or differs by 1, 2, or 3 nucleotides from, or is 1, 2, or 3 nucleotides longer or shorter at a 5' and/or 3' end than one of SEQ ID NOs:1-12, or 34-95.

[0113] One or more of the episomal vectors described herein can be provided as a kit for treatment of a disease in a mammalian subject associated with, exacerbated by, or caused by reduced transcription of a gene, reduced amount of a gene product, or reduced activity of a gene product. For example, an episomal vector encoding a CRISPR nuclease, a zinc finger nuclease, a TALEN, a TAL effector, a guide RNA, a transcriptional activation domain, a scaffold RNA, a scaffold RNA ligand, an affinity tag ligand, fusion proteins of one or more thereof, or a combination thereof, can be provided as a component of a kit containing an episomal vector packaging plasmid, cell line, or helper virus, or a combination thereof.

[0114] In some cases, an episomal vector in which the encoded polypeptide(s) and/or RNA(s) are flanked by AAV inverted terminal repeats is provided as a component of a kit containing additional materials for packaging the episomal vector into functional AAV particles. Such additional materials can include one or more plasmids encoding AAV rep and cap genes, one or more plasmids encoding adenovirus helper factors E1A, E1B, E2A, E4ORF6 and VA, adenovirus, or a combination thereof. In some cases, the trans-activing elements and/or helper elements for AAV packaging are provided in a stable cell line as a component of the kit.

[0115] In some embodiments, the cap gene is an AAV-DJ, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9 cap gene. In some embodiments, the cap gene is an AAV-DJ, AAV1, AAV2, AAV5, AAV7, AAV8 or AAV9 cap gene. In some embodiments, the cap gene is an AAV2 cap gene. In some embodiments, the cap gene is an AAV-DJ cap gene. In some embodiments, the inverted terminal repeats (ITRs) are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9 ITRs. In some embodiments, the ITRs are AAV1, AAV2, AAV5, AAV7, AAV8 or AAV9 ITRs. In some embodiments, the ITRs are AAV2 ITRs. In some cases, the capsid protein encoded by the cap gene is the same serotype as the ITRs. For

example, the cap gene can be an AAV2 cap gene and the ITRs can be AAV2 ITRs. In some cases, the capsid protein encoded by the cap gene is a different serotype from the serotype of the ITRs. Thus, for example, the cap gene can be an AAV5 cap gene and the ITRs can be AAV2 ITRs. As another example, the cap gene can be an AAV-DJ cap gene and the ITRs can be AAV2 ITRs.

[0116] In some cases, the episomal vector can be in a target cell or cell of the target tissue. In some cases, the target cell or cell of a target tissue is a dividing cell. In some cases, the cell is a non-dividing cell. In some cases, the cell is a neuron. In some cases, the cell is a cell of the hypothalamus. In some cases, the target cell or cell of the target tissue is a mammalian cell that contains a genome having at least one functional copy of a target gene, wherein the functional cop(y/ies) in the absence of transcriptional activation by a heterologous complex do not produce enough of a corresponding gene product to produce a wild-type phenotype in an organism. In some cases, the mammalian cell further comprises a scaffold RNA encoded by an episomal vector described herein, a guide RNA encoded by an episomal vector described herein, a CRISPR nuclease encoded by an episomal vector described herein, a SunTag encoded by an one or more episomal vectors described herein, a synergistic activation mediator (SAM) encoded by one or more episomal vectors described herein, a transcriptional activation domain encoded by an episomal vector described herein, an affinity tag ligand encoded by an episomal vector described herein, a fusion of one or more polypeptides described herein encoded by an episomal vector described herein, or a combination thereof.

[0117] In some cases, the episomal vector in a target cell or a cell of a target tissue is converted to a circular form, a circular concatemer, or a linear concatemer, *e.g.*, through recombination of repeat elements, such as ITRs. In some cases, the episomal vector in the target cell or the cell of a target tissue is converted from a single-stranded DNA vector into a double-stranded DNA. In some cases, the double-stranded DNA is converted into a circular form, a circular concatemer, or a linear concatemer. In some cases, the episomal vector in the target cell or cell of the target tissue persists as an episomal element providing persistent transgene (*e.g.*, CRISPR nuclease, transcriptional activator, guide RNA, scaffold RNA, *etc.*) expression. In some cases, the episomal elements is one of the foregoing circular forms, circular concatemers, or linear concatemers.

Viral Particles

[0118] One or more of the foregoing episomal vectors can be packaged in a viral particle. For example, the viral particle can contain an episomal vector encoding a CRISPR nuclease, a guide RNA, a scaffold RNA, a transcriptional activator, an affinity tag, an affinity tag ligand, a scaffold RNA ligand, a fusion protein of one or more thereof, or a combination of one or more thereof. The viral particle can be a viral particle that is capable of delivering the episomal vector to a target cell or tissue, such that the episomal vector enter the nucleus of a target cell or a cell of a target tissue and do not, or do not substantially integrate into the genome of the cell.

[0119] In some cases, the viral particle delivers the episomal vector to the target cell or cell of the target tissue and the episomal vector is converted to a circular form, a circular concatemer, or a linear concatemer, *e.g.*, through recombination of repeat elements, such as ITRs. In some cases, the episomal vector delivered by the viral particle is converted from a single-stranded DNA vector into a double-stranded DNA. In some cases, the double-stranded DNA is converted into a circular form, a circular concatemer, or a linear concatemer. In some cases, the viral particle delivers an episomal vector to a target cell or cell of the target tissue, and the episomal vector persists as an episomal element providing persistent transgene expression.

[0120] The viral particles can be EBV or AAV viral particles. In some cases, the viral particles are AAV viral particles. In some cases, the viral particles are AAV-DJ, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9 viral particles. In some cases, the viral particles are AAV-DJ, AAV1, AAV2, AAV5, AAV7, AAV8 or AAV9 viral particles. In some cases, the viral particles are AAV2 viral particles. . In some cases, the viral particles are AAV-DJ viral particles. The genome packed in the viral particle and encoding the one or more transgenes (the episomal vector) can be an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9 genome. In some cases, the genome is an AAV1, AAV2, AAV5, AAV7, AAV8 or AAV9 genome. In some cases, the genome is an AAV2 genome. In some cases the genome is the same serotype as the viral particle in which it is packaged. In other cases, the genome and viral particle are of different serotypes. For example, the capsid can be AAV5 serotype and the episomal vector can be AAV2 serotype. As another example, the capsid can be an AAV-DJ serotype and the episomal vector can be an AAV2 serotype.

[0121] One or more of the viral particles described herein can be provided as a kit for treatment of a disease in a mammalian subject associated with, exacerbated by, or caused by reduced transcription of a gene, reduced amount of a gene product, or reduced activity of a gene product. For example, an episomal vector encoding a CRISPR nuclease, a guide RNA, a transcriptional activation domain, a scaffold RNA, a scaffold RNA ligand, an affinity tag ligand, fusion proteins of one or more thereof, or a combination thereof, can be packaged into one or more viral particles and provided as a component of a kit containing a suitable pharmaceutical excipient, carrier, diluent, or buffer for delivery to a subject.

[0122] In one embodiment, the viral particles are in a suitable pharmaceutical excipient, carrier, diluent, or buffer for delivery to a subject. Such excipients, carriers, diluents, and buffers include any pharmaceutical agent that can be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not limited to, liquids such as water, saline, glycerol and ethanol. Pharmaceutically acceptable salts can be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. A wide variety of pharmaceutically acceptable excipients are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th edition, Lippincott, Williams, & Wilkins Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H. C. Ansel et al., eds., 7th ed., Lippincott, Williams, & Wilkins and Handbook of Pharmaceutical Excipients (2000) A. H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

Methods

[0123] Described herein are methods for treating a disease in a mammalian subject associated with, exacerbated by, or caused by reduced transcription of a gene, reduced amount of a gene product, or reduced activity of a gene product by increasing transcription of a target gene. The methods generally include contacting a target cell or a cell of a target tissue with one or more of the foregoing episomal vectors. In some embodiments, the episomal vectors are non-integrating

or substantially non-integrating. In some embodiments, the episomal vectors are packaged into viral particles and the viral particles are contacted with the target cell or the cell of a target tissue. In some cases, the contacting is performed *in vivo*. In some cases, the contacting is performed *in vitro* (*e.g.*, using primary cells obtained from the subject) and the contacted cells are delivered to a subject, or optionally cultured and delivered to the subject.

[0124] The episomal vectors (*e.g.*, packaged into viral particles) can be delivered by any means known in the art. In some cases, the episomal vectors are contacted with a cell *in vivo* by systemic delivery (*e.g.*, intravenous delivery). In some cases, the episomal vectors (*e.g.*, packaged into viral particles) are contacted with a cell *in vivo* by site-specific delivery to an affected cell or tissue. For example, viral particles in which episomal vectors are packaged can be injected into a site of an affected cell or tissue. In some cases, two or more episomal vectors are packaged into viral particles such that each viral particle contains a single copy of one of the two or more episomal vectors or is empty (contains no genome or a genome that lacks a functional transgene). Such viral particles can be delivered as a mixture or individually. In some cases, the particles are delivered simultaneously. In some cases, the particles are delivered sequentially. Typically, the particles are delivered such that the delivered transgenes encoded by the episomal vectors are co-expressed in the subject such that a disease is treated.

[0125] In one embodiment, one or more different viral particles (*e.g.*, viral particles having the same capsid but containing vectors that encode different transgenes) are injected into a brain of a subject. In some cases, the one or more viral particles are injected into a hypothalamus of a subject. The viral particles can be delivered to an anterior portion of the hypothalamus, a posterior portion of the hypothalamus, a ventromedial portion of the hypothalamus, or a combination thereof. The viral particles can be delivered bilaterally (*e.g.*, via bilateral injections to a hypothalamus of a subject). In some cases, the one or more viral particles are delivered to a neuron of the subject. In some case, the one or more viral particles are delivered by stereotactic injection.

[0126] The dose of viral particle delivered to a subject can be from $1x10^3$ viral particles/kg subject to $1x10^{20}$ viral particles/kg subject. The dose of episomal vector delivered to a subject can be from $1x10^3$ vector molecules/kg subject to $1x10^{20}$ vector molecules/kg subject. In some cases, the dose is from $1x10^4$ to $1x10^{18}$, from $1x10^5$ to $1x10^{16}$, from $1x10^6$ to $1x10^{15}$ viral

particles/kg subject or vector molecules/kg subject. In some cases, the dose is at least $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, or $1x10^{15}$ viral particles/kg subject or vector molecules/kg subject. In some cases, vector molecules are in the form of viral genomes delivered in a viral particle. In some cases, the dose is a dose of delivered viral genome (*e.g.*, packaged in a viral particle) encoding a CRISPR nuclease (*e.g.*, dCas9 fused to an activation domain) and a guide RNA (*e.g.*, sgRNA). In some cases, the dose is a dose of delivered viral genome (*e.g.*, packaged in a viral particle) encoding a CRISPR nuclease (*e.g.*, dCas9 fused to an activation domain), and a second dose, such as one or more of the foregoing doses is a dose of delivered viral genome (*e.g.*, packaged in a viral particle) encoding guide RNA (*e.g.*, sgRNA).

[0127] In some cases, a systemic does can be higher as compared to a dose applied directly to a tissue or organ to be treated. For example, for treatment of obesity dysregulated by a haploinsufficient sim1 gene in hypothalamus tissue or cell, a lower dose can be delivered to the hypothalamus as compared to a systemic dose. In humans, systemic delivery can, *e.g.*, be about 6.7×10^{13}- 2.0×10^{14} viral genomes (vg)/kg (see, clinicaltrials.gov/ct2/show/NCT02122952) and neurosurgical delivery can, *e.g.*, be about 7.5×10^{11}- 8.8×10^{12} vg/kg (see clinicaltrials.gov/ct2/show/NCT01973543).

[0128] A dose can be administered once, or multiple times. In some cases, the dose is delivered at least once within a period of 30 days, 60 days, 90 days, 120 days, or 180 days. In some cases, a dose is delivered at least once every 10 weeks, 20 weeks, 30 weeks, 40 weeks, 52 weeks, or 75 weeks, or 100 weeks. In some cases, a dose is delivered at least once every 6 months, 12 months, 18 months, 2 years, 3 years, 5 years, or 10 years. In some cases, a single dose or 2, or 3, or 4 doses results in persistent and sufficient expression of the otherwise haploinsufficient target gene to treat at least one symptom of a disease or condition caused by the haploinsufficiency for a period of months or years. In some cases, a dose is administered, the sufficiency of expression of a target haploinsufficient gene (*e.g.*, a gene in Table 1 such as sim1) is assessed (*e.g.*, in a target tissue such as hypothalamus) and additional doses are delivered as needed by the same or different route. In some cases, one or more doses of viral particles as described herein are delivered, in sufficient amount to increase transcription of a target gene and thereby treat at least one symptom of a disease associated with, exacerbated by, or caused by

reduced transcription of a gene, reduced amount of a gene product, or reduced activity of a gene product, and one or more doses are re-administered when transcription of the target gene has reduced from its maximal expression by at least 10%, 25%, 50%, 75%, 90%, or more.

EXAMPLES

Rescue of Haploinsufficiency-caused Obesity

I. Introduction

[0129] Over 300 genes are known to cause human disease due to haploinsufficiency (1, 2), leading to a wide range of phenotypes that include cancer, neurological diseases, developmental disorders, immunological diseases, metabolic disorders, infertility, kidney disease, limb malformations and many others (1). Large-scale exome sequencing analyses estimate that a total of 3,230 human genes could be heterozygous loss-of-function (LoF) intolerant (3). Gene therapy holds great promise in correcting haploinsufficient diseases, by inserting a functional recombinant copy or copies of the mutant gene. Currently, there are a total of 2,300 clinical trials underway for gene therapy, the majority of them using adeno-associated virus (AAV) to deliver the recombinant gene (4). AAV is a preferred gene delivery method due to its ability to deliver DNA without integrating into the genome, not causing pathogenicity and providing long lasting gene expression of the transgene (5). However, AAV has an optimal 4.7 kilo base (kb) packaging capacity, limiting its gene therapy use for genes longer than 3.5kb (taking into account additional regulatory sequences needed for its stable expression). Analysis of the 3,230 heterozygous LoF genes finds 715 (22%) of them to have coding sequence longer than 3.5kb, rendering them not suitable for AAV gene therapy.

[0130] CRISPR gene editing can potentially fix haploinsufficient mutations, however it would require the need to custom tailor the editing strategy for each mutation. Moreover, it's not a feasible therapy for heterozygous LoF micro-deletions. To address these challenges, we devised a novel therapeutic strategy for haploinsufficiency using CRISPR activation (CRISPRa). CRISPRa takes advantage of the RNA-guided targeting ability of CRISPR to direct a nuclease deficient Cas9 (dCas9) along with a transcriptional activator to regulatory element/s of a specific gene, thus increasing its expression (6-10). Here, we tested whether we can use this system to increase the transcription of the unaffected endogenous gene in a haploinsufficient disease to rescue the disease phenotype.

[0131] *SIM1* is a transcription factor that is expressed in the developing kidney and central nervous system, and is essential for the formation of the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus (11). It is also thought to play a major role in the leptin pathway (12). In humans, haploinsufficiency of *SIM1* due to chromosomal aberrations (12, 13) results in hyperphagic obesity (13) and *SIM1* coding mutations, many of them being loss-of-function, are thought to be a major cause of severe obesity in humans (14-16). *Sim1* homozygous null mice die perinatally, while *Sim1* heterozygous mice ($Sim1^{+/-}$) survive, are hyperphagic and develop early-onset obesity with increased linear growth, hyperinsulinemia and hyperleptinemia (17). A postnatal conditional knockout of hypothalamic *Sim1* leads to a similar phenotype in heterozygous mice (18), implicating *Sim1* to be an important regulator of energy homeostasis. Overexpression of *SIM1*, using a human bacterial artificial chromosome in mice, rescues diet-induced obesity and reduced food intake (19), suggesting a potential role for *Sim1* as a general therapeutic target for obesity. Here, we used *Sim1* as our proof of concept model for our CRISPRa therapeutic strategy. We tested the ability of CRISPRa to rescue the obesity phenotype in $Sim1^{+/-}$ mice using both transgenic and AAV based approaches targeting the *Sim1* promoter or its hypothalamus specific enhancer. Our results present a novel therapeutic approach for treating haploinsufficient diseases, or other diseases caused by altered gene dosage.

II. Results

A. Upregulation of *Sim1 in vitro*

[0132] We first set out to optimize our CRISPRa conditions *in vitro*. *SIM1* has a well characterized promoter (20) and distant hypothalamus enhancer (~270kb from the transcription start site), *Sim1* candidate enhancer 2 (SCE2 (21)), both of which were chosen as targets for CRISPRa **(Fig. 1A)**. We designed sgRNAs for either the *Sim1* promoter or enhancer (SCE2). Using these guides we tested if dCas9 fused to VP64 (dCas9-VP64), a transcriptional activator that carries four tandem copies of VP16 (a herpes simplex virus type 1 transcription factor) (22), can overexpress *Sim1* in mouse neuroblastoma cells (Neuro-2a). This activator was chosen due to its lower activation levels compared to other known activators (23), as we wanted to obtain therapeutic *Sim1* dosage levels *in vivo* that are similar to wild-type. Cells were transfected with dCas9-VP64 and the various guides and following 48 hours *Sim1* mRNA levels were measured using quantitative PCR (qPCR). We identified one sgRNA for either promoter or SCE2 that was able to overexpress endogenous *Sim1* by 13 and 4 fold respectively **(Fig. 1B).** Additionally, we

identified four sgRNAs for the *Sim1* promoter that were able to overexpress endogenous *Sim1* by over 4-fold (**Fig. 7A**) and at least one sgRNA for SCE2 that was able to overexpress endogenous *Sim1* by over 2-fold (**FIG. 8A**).

B. Transgenic CRISPRa rescues obesity

[0133] To test the ability of our CRISPRa system to activate *Sim1 in vivo*, we generated knockin mouse lines using TARGATT technology (24) that have dCas9-VP64 inserted into the mouse *Hipp11* ($H11P^{CAG\text{-}dCas9\text{-}VP64}$) locus and either sgRNA, targeting the *Sim1* promoter ($ROSA26^{Sim1Pr\text{-}sgRNA}$) or SCE2 ($ROSA26^{SCE2En\text{-}sgRNA}$), in the *Rosa26* locus (**Fig. 1C**). We then crossed these mice to $Sim1^{+/-}$ mice that develop severe obesity (17). Mice having all three alleles ($Sim1^{+/-}$ X $H11P^{CAG\text{-}dCas9\text{-}VP64}$ and $ROSA26^{Sim1Pr\text{-}sgRNA}$ or $ROSA26^{SCE2En\text{-}sgRNA}$) were maintained using breeders chow (picodiet-5058) and weighed on a weekly basis until 16 weeks of age along with wild-type littermates and $Sim1^{+/-}$ X $H11P^{CAG\text{-}dCas9\text{-}VP64}$ mice and $Sim1^{+/-}$, both of which become severely obese (negative controls). Analysis of at least seven females and seven males per condition showed that $Sim1^{+/-}$ mice carrying both dCas9-VP64 and either *Sim1* promoter or enhancer sgRNA have a significant reduction in body weight compared to $Sim1^{+/-}$ X $H11P^{CAG\text{-}dCas9\text{-}VP64}$ and $Sim1^{+/-}$ littermates (**Figs. 1D-F**).

C. CRISPRa corrects $Sim1^{+/-}$ metabolic profile

[0134] To relate body weight reduction with body composition and metabolic parameters, we next performed metabolic profiling for $Sim1^{+/-}$ X $H11P^{CAG\text{-}dCas9\text{-}VP64}$ X $ROSA26^{Sim1Pr\text{-}sgRNA}$ (Prm CRISPRa) $Sim1^{+/-}$ X $H11P^{CAG\text{-}dCas9\text{-}VP64}$ X $ROSA26^{SCE2En\text{-}sgRNA}$ (Enh-CRISPRa) and our other mouse lines. Three mice for each genotype were analyzed for body composition and metabolic profiling, right at the onset of the obesity phase, 6-8 weeks of age. Both Prm-CRISPRa and Enh-CRISPRa mice showed a significant reduction in body fat content compared to $Sim1^{+/-}$ in both females and males (**Fig. 2A**). Metabolic chamber analyses of other hallmarks of $Sim1^{+/-}$ obese mice such as oxygen consumption and food intake showed a shift towards wild-type metabolic parameters in the Prm-CRISPRa and Enh-CRISPRa mice (**Fig. 2B-C**). In addition, their respiratory exchange ratio (RER; VCO2/VO2), an indirect method of defining basic metabolic rate, also showed parameters similar to their wild-type littermates (**Fig. 2D**). However, we did not observe any significant differences for their physical activity in individual chambers. Combined, these results show that both Prm-CRISPRa and Enh-CRISPRa mice have less body

fat and demonstrate an improvement in their metabolic parameters that contribute towards a reduction in their overall body weight.

D. *Sim1* activation is tissue-specific

[0135] To test for *Sim1* activation levels and tissue-specificity in our mice, we measured its mRNA expression levels in different tissues. We selected two tissues where *Sim1* is known to be expressed, hypothalamus and kidney, and two tissues where it is not expressed, lung and liver (25) **(Fig. 3A).** We first measured *dCas9* expression, and found it to be expressed in all four tissues, as expected, since we used a ubiquitous CMV enhancer chicken beta-Actin (CAG) promoter to drive its expression (**Fig. 3B**). In contrast, for *Sim1*, we observed significantly higher mRNA levels in the hypothalamus and kidney in Prm-CRISPRa mice and only in the hypothalamus of Enh-CRISPRa mice compared to $Sim1^{+/-}$ mice (**Fig. 3C-D**). Since we did not observe any significant differences between the obesity phenotype of Prm-CRISPRa and Enh-CRISPRa mice, we could speculate that the activation of *Sim1* in the hypothalamus is sufficient to rescue the $Sim1^{+/-}$ obesity phenotype. Interestingly, in tissues where *Sim1* is not expressed (*i.e.* liver and lung), we could not detect *Sim1* expression in Prm-CRISPRa or Enh-CRISPRa mice despite observing Cas9 expression. These results imply that in the *in vivo* conditions of our study, dCas9-VP64 could only upregulate expression in tissues where the *cis*-regulatory elements of its target gene are active. This suggests that *cis*-regulatory elements could be used to determine the tissue-specificity of CRISPRa.

E. CRISPRa AAV reduces $Sim1^{+/-}$ weight gain

[0136] To further translate this approach to a therapeutic strategy for haploinsufficiency, we took advantage of AAV to deliver CRISPRa into the hypothalamus of $Sim1^{+/-}$ mice. We generated the following three AAV vectors: 1) dCas9-VP64 driven by a cytomegalovirus (CMV) promoter (*pCMV-dCas9-VP64*); 2) *Sim1* promoter sgRNA along with mCherry (*pU6-Sim1Pr-CMV-mCherry*); 3) SCE2 sgRNA along with mCherry (*pU6-SCE2-CMV-mCherry*). For the *pCMV-dCas9-VP64* vector, due to the size of dCas9-VP64 expression cassette, we obtained a 5.4kb insert. While this insert size is above the 4.7kb limit, it was shown that going above 5kb reduces transgene expression levels but still could be used for delivery (26). These vectors were packaged individually into AAV-DJ serotype, which is a chimera of type 2, 8 and 9 that was shown to achieve high expression levels in multiple tissues (27) **(Fig. 4A)**. We did observe lower

but usable viral titers for *pCMV-dCas9-VP64* AAV (see methods). We first tested if of our AAV CRISPRa vectors could overexpress *Sim1 in vitro* using Neuro-2a cells. We observed a 4 and 5 fold upregulation of *Sim1* mRNA expression when targeting the promoter or enhancer respectively (**Fig. 4A**). Using additional sgRNAs (SEQ ID NOS:38, 40 or 42), we observed that our AAV CRISPRa vectors could overexpress *Sim1 in vitro* using Neuro-2a cells. We observed a 2-fold to 6-fold upregulation of *Sim1* mRNA expression when targeting the promoter (**Fig. 7B**) and a 2-fold to 4.5-fold upregulation of *Sim1* mRNA expression when targeting the enhancer (SCE2) (**Fig. 8B**).

[0137] Next, we performed stereotactic injections to deliver virus carrying *pCMV-dCas9-VP64* and either *pU6-Sim1Pr-CMV-mCherry* (Prm-CRISPRa-AAV) or *pU6-SCE2-CMV-mCherry* (Enh-CRISPRa-AAV) into the PVN of the hypothalamus of *Sim1*$^{+/-}$ mice at four weeks of age, before they start developing obesity. As negative controls, we also injected *Sim1*$^{+/-}$ mice with *pCMV-dCas9-VP64* virus only. We tested for the expression of our sgRNA-CMV-mCherry cassette by performing immunostaining on the hypothalamus of injected mice and found it to be expressed in the PVN (**Fig. 4B-C**). To test whether *Sim1* expression levels were increased by delivering CRISPRa-AAV to the hypothalamus of *Sim1*$^{+/-}$ mice, we measured mRNA expression levels for both *dCas9* and *Sim1* from 11 week old AAV injected mice. *dCas9* was found to be expressed in the hypothalamus of all our *pCMV-dCas9-VP64* AAV injected mice (**Fig. 4D**). *Sim1* upregulation was observed in both Prm-CRISPRa-AAV and Enh-CRISPRa-AAV injected hypothalami, but not in mice injected with only *pCMV-dCas9-VP64*-AAV (**Fig. 4E**). The injected mice were measured for body weight up to 11 weeks of age (**Fig. 5A**). We observed a significant weight reduction in the Prm-CRISPRa-AAV or Enh-CRISPRa-AAV injected mice compared to the *Sim1*$^{+/-}$ or *pCMV-dCas9-VP64*-AAV injected *Sim1*$^{+/-}$ mice (**Fig, 5B-C**). These results show that CRISPRa-AAV mediated upregulation could be used as a viable gene therapy tool to treat haploinsufficiency.

F. Upregulation of *Mc4r in vitro*

[0138] Over 70% of obesity that has genetic basis is caused by defects in the leptin pathway. *MC4R* is part of the leptin pathway and mutations in it are the most commonly found mutations in obese individuals (~5% of the 1 percentile obese population). Since it is a downstream factor, upregulation of *MC4R* and *SIM1* could possibly rescue obesity caused by mutations in these

other leptin pathway genes. Here, we have shown that we can upregulate *MC4R* by targeting its promoter and have also shown that upregulation of *SIM1* can increase *MC4R* expression. We were also able to rescue the obesity phenotype in Mc4r heterozygos mice (performed essentially as set forth in the upregulation of *Sim1 in vitro*, discussed above). As such, *MC4R* upregulation could be used as therapy for obesity.

[0139] We designed sgRNAs for the *Mc4r* promoter (See, SEQ ID NOS:50-54). Using these guides we tested if dCas9 fused to VP64 (dCas9-VP64) can overexpress *Mc4r* in mouse neuroblastoma cells (Neuro-2a). Cells were transfected with dCas9-VP64 and the various guides and following 48 hours *Mc4r* mRNA levels were measured using quantitative PCR (qPCR). We identified one sgRNA for the *Mc4r* promoter that was able to overexpress endogenous *Mc4r* by 7-fold (**Fig. 9A**).

G. CRISPRa AAV induces upregulation of *Mc4r*

[0140] We next tested if of our AAV CRISPRa vectors (prepared essentially as described under *Sim1* CRISPRa AAV, above) containing sgRNAs, SEQ ID NOS:51, 52 or 54, could overexpress *Mc4r in vitro* using Neuro-2a cells. We observed between a 3.4-fold and 6.6-fold upregulation of *Mc4r* mRNA expression when targeting the promoter (**Fig. 9B**).

H. Upregulation of *SCN2A in vitro*

[0141] Mutations in *SCN2A* are the most commonly found mutations in individuals with autism spectrum disorder (ASD) and epilepsy. The majority of mutations are loss of function leading to ASD due to haploinsufficiency. Here, we have shown that we can upregulate *SCN2A* by targeting its promoter. As such, SCN2A upregulation could be used as therapy for ASD and epilepsy.

[0142] We designed sgRNAs for the *Scn2a* promoter (See, SEQ ID NOS:85-91). Using these guides we tested if dCas9 fused to VP64 (dCas9-VP64) can overexpress *Scn2a* in mouse neuroblastoma cells (Neuro-2a). Cells were transfected with dCas9-VP64 and the various guides and following 48 hours *Scn2a* mRNA levels were measured using quantitative PCR (qPCR). We identified four sgRNAs for the *Scn2a* promoter that were able to overexpress endogenous *Scn2a* by over 2-fold (**Fig. 12A**).

I. CRISPRa AAV induces upregulation of *Scn2A*

[0143] We next tested if of our AAV CRISPRa vectors (prepared essentially as described under *Sim1* CRISPRa AAV, above) containing sgRNAs, SEQ ID NOS:92-94, could overexpress *Scn2a in vitro* using Neuro-2a cells. Two different multiplicity of infection (MOI) were used: 5,000 and 1,750 viral genome (vg/ml). We observed a slight upregulation of *Scn2a* mRNA expression when targeting the promoter with a MOI of 5,000 viral genomes per ml (**Fig. 12B**).

J. Upregulation of *SETD5 in vitro*

[0144] Mutations in *SETD5* lead to mental retardation-23 (OMIM #615761) which include intellectual disability and dysmorphic features. Here, we have shown that we can upregulate *SETD5* by targeting its promoter. As such, SETD5 upregulation could be used as therapy for intellectual disability.

[0145] We designed sgRNAs for the *Setd5* promoter (See, SEQ ID NOS:75-84). Using these guides we tested if dCas9 fused to VP64 (dCas9-VP64) can overexpress *Setd5* in mouse neuroblastoma cells (Neuro-2a). Cells were transfected with dCas9-VP64 and the various guides and following 48 hours *Setd5* mRNA levels were measured using quantitative PCR (qPCR). We identified two sgRNAs for the *Setd5* promoter that were able to overexpress endogenous *Setd5* by over 1.5-fold (**Fig. 11B**).

[0146] Next, we designed sgRNAs for the *SETD5* promoter in humans (See, SEQ ID NOS:65-74). Using these guides we tested if dCas9 fused to VP64 (dCas9-VP64) can overexpress *SETD5* in human HEK293T cells. Cells were transfected with dCas9-VP64 and the various guides and following 48 hours *SETD5* mRNA levels were measured using quantitative PCR (qPCR). We identified at least one sgRNA for the *SETD5* promoter that was able to overexpress endogenous *SETD5* by over 2.5-fold (**Fig. 11A**).

K. Upregulation of *PKD1 in vitro*

[0147] Mutations in *PKD1* lead to autosomal dominant polycystic kidney disease (ADPKD; OMIM #173900) which is the most frequent hereditary kidney disorder affecting 1 to 400-1000 individuals. 85% of ADPKD is caused by mutations in *PKD1*, the majority of which are loss-of-function. *PKD1* is 13kb long and as such cannot be packaged in standard gene therapy vectors. Using the CRISPRa technology disclosed herein, we have shown that we can upregulate *PKD1*

by targeting its promoter. As such, PKD1 upregulation could be used as therapy for autosomal dominant polycystic kidney disease.

[0148] We designed sgRNAs for the *PKD1* promoter in humans (See, SEQ ID NOS:55-64). Using these guides we tested if dCas9 fused to VP64 (dCas9-VP64) can overexpress *PKD1* in human HEK293T cells. Cells were transfected with dCas9-VP64 and the various guides and following 48 hours *PKD1* mRNA levels were measured using quantitative PCR (qPCR). We identified at least three sgRNAs for the *PKD1* promoter that were able to overexpress endogenous *PKD1* by over 2-fold (**Fig. 10**).

L. Upregulation of *PAX6 in vitro*

[0149] Loss-of-function mutations in *PAX6* lead to Aniridia 1 (OMIM #106210) due to haploinsufficiency. Here, we have shown that we can upregulate *PAX6* by targeting its promoter. As such, PAX6 upregulation could be used as therapy for aniridia 1.

[0150] We designed one sgRNA for the *PAX6* promoter in humans (SEQ ID NO:95). Using this guide we tested if dCas9 (*S. pyogenes*) fused to VP64 (dCas9-VP64) can overexpress *PAX6* in Human H1-ESC cells differentiated into neurons. Cells were infected with lentivirus carrying the guide, and following 48 hours *PAX6* mRNA levels were measured using quantitative PCR (qPCR). Our exemplary sgRNA for the *PAX6* promoter was able to overexpress endogenous *PAX6* by over 6-fold (**Fig. 13**). Fig. 13 also demonstrates that additional neuronal markers (e.g., NES) were also capable of neural induction of H1-ESCs.***III. Discussion***

[0151] CRISPR-based gene editing is a promising therapeutic technology to correct genetic mutations. However, it currently is not a feasible technology for haploinsufficiency, limited by low non-homologous end joining (NHEJ) efficiencies (*i.e.* editing only a small portion of cells) and the need to custom tailor specific guides and donor sequences for each individual mutation. In addition, it is not a feasible therapeutic strategy for micro-deletions, over 200 of which are known to cause human disease (28), primarily due to haploinsufficiency. In this study, we used a novel approach to tackle these hurdles and show how a haploinsufficient disease could be corrected by increasing the transcriptional output from the existing functional allele via CRISPRa.

[0152] Using CRISPRa targeting for either the promoter or enhancer of *Sim1*, we were able to rescue the obesity phenotype in a tissue-specific manner in mice that are haploinsufficient for

Sim1 **(Fig. 6)**. As this therapeutic approach takes advantage of the existing functional allele, it has several benefits: 1)It overcomes the need to custom tailor CRISPR gene editing approaches for different haploinssufficient causing mutations in the same gene. 2) This approach could potentially be used to target two or more genes. As such, it could pose as a potential therapeutic strategy for micro-deletions related-diseases that are caused by the heterozygous LoF of more than one gene. 3) CRISPRa-AAV could be used to rescue haploinsufficient diseases caused by genes that are longer than its optimal packaging capability. 4) CRISPR-based therapies can take advantage of *cis*-regulatory elements to guide tissue-specificity. The availability of large-scale tissue-specific maps of gene regulatory elements could provide ample candidates to use for this therapeutic approach. We observed distinct difference in tissue specific activation of *Sim1* in our study, which can be attributed to chromatin accessibility of the locus in various tissues. Previous large-scale Cas9 and dCas9 cell culture screens have shown a targeting preference for regions with low nucleosome occupancy (29). Active promoters or enhancers would have lower nucleosome occupancy, thus being more amenable to dCas9 targeting.

[0153] Our dCas9-VP64 mouse and AAV vectors can be a useful tool for targeted gene activation *in vivo* by delivering sgRNA/s targeted to a specific gene/s in certain tissues/cell types. This approach could be used to assess gene-gene interactions or for the identification of the target gene/s of a specific regulatory element *in vivo* by measuring its expression level following activation. Another potential area of study could be neuronal circuit manipulation. Discrepancies between acute and chronic neuronal circuit manipulations have been observed (30) which can be addressed by our AAV-CRISPRa and Transgenic-CRISPRa strategies respectively.

[0154] Haploinsufficiency of *Sim1* causes obesity both in mice (17) and humans (13). Whether this is caused by the reduction in PVN size during development that is observed in $Sim1^{+/-}$ mice (17) or by disturbed energy homeostasis during adulthood was an area of major research. The obesity phenotype observed in the postnatal conditional knockout of hypothalamic *Sim1* (18), reinforced the hypothesis that *Sim1* does indeed have a role in energy homeostasis later during adulthood. Our ability to rescue the obesity phenotype via CRISPRa AAV injections into the hypothalamus of 4 week old mice, further corroborates this role. Abrogation of melanocortin 4 receptor (*Mc4r*) signaling is the hallmark of most polygenic and monogeneic obesity phenotypes. Conditional postnatal deficiency of *Sim1* leads to reduced levels of Mc4r signaling. As *Sim1* was

shown to be an integral downstream component of the leptin-Mc4r pathway (18), *Sim1* CRISPRa targeting could provide a potential therapy for conditions that disrupt the leptin signaling pathway.

[0155] Despite technological advances in CRISPR-based therapeutic intervention, our understanding of the long-term side effects of CRISPR expression and its off-targeting effects *in-vivo* still remains largely unknown, which also holds true for our current study. Anti-CRISPR genes (31) or conditional activation or silencing of our CRISPRa system could be able to address these concerns in future. Furthermore, there is also a need to develop CRISPRa/i tools to modulate gene dosage, so as to be able to optimize transcriptional output for certain diseases where higher or lower activation levels might be needed. In this study, we used VP64 as our activator, due to its known weak activation capacity (23) which fit with our need to obtain levels of gene expression that are similar to having two normal alleles. CRISPRa based gene activation is dependent upon the nature of the fused activator (23), sgRNA target (29) and may require optimization of the CRISPR system and delivery method.

[0156] As demonstrated in this study, CRISPRa can be used to activate genes not only by targeting their promoters, but by also targeting distal *cis*-regulatory elements such as enhancers. Previous studies have shown that these elements can be viable therapeutic targets. For example, by targeting a globin enhancer with zinc finger nucleases fused to a chromatin looping factor, the LIM domain binding 1 (*LDB1*) gene, activation of fetal hemoglobin was achieved *in vitro*, providing a potential therapy for sickle cell disease (37). In another study, re-activation of fetal hemoglobin was achieved by deactivating the enhancer of its repressor B-cell CLL/lymphoma 11A (*BCL11A*) using CRISPR gene editing (38). Our study provides a novel approach that also takes advantage of *cis*-regulatory elements for therapeutic purposes. There are numerous diseases that are caused by lower gene dosage that could potentially be treated with CRISPRa therapy. In addition, several human diseases could potentially be rescued by the activation of another gene with a similar function. These could include for example *Utrophin* for Duchenne Muscular Dystrophy (39), survival of motor neuron 2 (*SMA2*) for Spinal Muscular Atrophy (SMA; (40)) or the aforementioned fetal globin for sickle cell disease. Further development of this technology could provide a viable therapy for patients inflicted with these diseases.

III. Materials and Methods

Plasmids

[0157] The *pMSCV-LTR-dCas9-VP64-BFP* vector, encoding a mammalian codon-optimized *Streptococcus pyogenes* dCas9 fused to two C-terminal SV40 NLSs and tagBFP along with a VP64 domain and the U6-sgRNA-CMV-mCherry-T2A-Puro plasmids were used for cell line transfections (both kind gifts from Dr. Stanley Qi). sgRNAs were cloned using the In-Fusion HD-cloning kit (Clontech) following the manufacturer's protocol into the *BstX*I and *Xho*I sites. Mouse knockin vectors were generated by cloning dCas9-VP64 and U6-sgRNA-CMV-mCherry expression cassettes from the aforementioned vectors into the TARGATT (CAG + Poly A) plasmid (Applied StemCell). *pcDNA-dCas9-VP64* (Addgene 47107), and U6-sgRNA-CMV-mCherry-WPREpA were cloned replacing the *Ef1a-FAS-hChR2(H134R)-mCherry-WPRE-pA* with that of our U6-sgRNA-CMV-mCherry-WPREpA into the backbone of *pAAV-Ef1a-FAS-hChR2(H134R)-mCherry-WPRE-pA* (Addgene 37090).

AAV production

[0158] AAV DJ serotype particles were produced using the Stanford Neuroscience viral vector core. The packaging load for *pCMV-dCas9-VP64* was 5.4kb and for *pU6-Sim1Pr-CMV-mCherry* and *pU6-SCE2-CMV-mCherry* 2.5kb. Genomic titers were ascertained by WPRE and ITR probes to be $1.40E^{10}$ viral genome (vg)/ml for *pCMV-dCas9-VP64* and around $3.30E^{13}$ vg/ml for *pU6-Sim1Pr-CMV-mCherry* and 2.20 E^{13} vg/ml for *pU6-SCE2-CMV-mCherry* .

Cell culture

[0159] Neuroblastoma 2a cells (Neuro-2a; ATCC® CCL-131) were grown following ATCC guidelines. Plasmids were transfected into Neuro-2a cells using X-tremeGENE HP DNA transfection reagent (Roche) following the manufacturer's protocol. AAV particles were infected into Neuro2a cells at different MOIs. Neuro2a cells were harvested 48 hours post transfection and 5 days post infection to isolate RNA for qRT-PCR analysis.

[0160] Human HEK293T cells were grown following ATCC guidelines. Plasmids were transfected into these cells using X-tremeGENE HP DNA transfection reagent (Roche) following the manufacturer's protocol.

Quantitative reverse-transcription PCR

[0161] RNA was isolated from cells or tissues using RNeasy Mini Kit (Qiagen) following the manufacturer's protocol. For mice, animals were euthanized and tissues were harvested directly into the RNA lysis buffer of the RNeasy Mini Kit. The hypothalamus was dissected using a mouse Brain Matrix and slicers from Zivic Instruments. cDNA was prepared using SuperScript III First-Strand Synthesis System (Invitrogen) using the manufacturer's protocol along with DNaseI digestion. qPCR was performed using SsoFast EvaGreen Supermix (Biorad). The results were expressed as fold-increase mRNA expression of the gene of interest normalized to either beta-actin, *Rpl38* or *Elf3* expression by the ΔΔCT method followed by ANOVA and Tukey test for statistical analysis. Reported values are the mean and standard error of the mean from three independent experiments performed on different days (N=3) with technical duplicates that were averaged for each experiment.

Mice

[0162] *Sim1*$^{+/-}$ mice (17) on a mixed genetic background were obtained as a kind gift from Dr. Jacques Michaud lab. In these mice, a 1kb fragment containing 750bp of the 5' region, the initiation codon, and the sequence coding for the basic domain (the first 17 amino acids) was replaced by a *Pgk–neo* cassette, that was used for genotyping using KAPA mouse genotyping kit (KAPA Biosystems). To generate dCas9-VP64 and sgRNA mice we used TARGATT technology (24). DNA for injection was prepared and purified as mini-circles using the TARGATT Transgenic Kit, V6 (Applied StemCell). The injection mix contained 3 ng/μL DNA and 48 ng/μL of *in vitro* transcribed φC31o mRNA in microinjection TE buffer (0.1 mM EDTA, 10 mM Tris, pH 7.5) and injections were done using standard mouse transgenic protocols (41). dCas9-VP64 was inserted into the mouse *Hipp11* locus and sgRNAs into the *Rosa26* locus. Mice were genotyped using the using the KAPA mouse genotyping kit. F0 TARGATT knock-ins were assessed using PCR7+8, PCR1 described in (PMID: 21464299) along with vector insertion specific dCas9-VP64 primers as well as mCherry specific primers. All mice were fed *ad libitum* Picolab mouse diet 20, 5058 containing 20% protein, 9% fat, 4% fibre for whole study. Calories provided by: Protein, % 23.210 Fat (ether extract), % 21.559 Carbohydrates, % 55.231. All animal work was approved by the UCSF Institutional Animal Care and Use Committee.

Mouse body weight measurements.

[0163] *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$, *ROSA26*$^{Sim1Pr\text{-}sgRNA}$ and *ROSA26*$^{SCE2En\text{-}sgRNA}$ mice were mated with FVB mice for 3-5 generations to assess germline transmission. Three independent integrants were used from each line to set up matings. *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ were mated with *Sim1*$^{+/-}$ and subsequent *Sim1*$^{+/-}$ X *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ mice were rossed with either *ROSA26*$^{Sim1Pr\text{-}sgRNA}$ or *ROSA26*$^{SCE2En\text{-}sgRNA}$ to generate mice having all three unlinked alleles. Mice were maintained at Picodiet 5058 throughout the study and at least 6 females and 6 males from all genotypes (wild-type littermates, *Sim1*$^{+/-}$, *Sim1*$^{+/-}$ X *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$, *Sim1*$^{+/-}$ X *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{Sim1Pr\text{-}sgRNA}$, *Sim1*$^{+/-}$ X *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{SCE2En\text{-}sgRNA}$) were measured for their body weights from 4-16 weeks of age on a weekly basis.

Mouse metabolic profiling

[0164] Metabolic rates from individual mice were measured using the Columbus Instruments Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments). Mice were single housed and acclimatized on powdered picodiet 5058 for 3-4 days before performing the metabolic monitoring. We individually housed mice in CLAMS units and measurements were carried out over 4-5 days. The temperature was maintained at 22°C and oxygen and carbon dioxide were calibrated with 'Air reference' set at 20.901 and 0.0049. Three males and three females from each genotype: wild-type littermates, *Sim1*$^{+/-}$, *Sim1*$^{+/-}$ X *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{Sim1Pr\text{-}sgRNA}$, *Sim1*$^{+/-}$ X *H11P*$^{CAG\text{-}dCas9\text{-}VP64}$ X *ROSA26*$^{SCE2En\text{-}sgRNA}$ were measured. with metabolic parameter (VCO2, VO2, RER, food intake, and activity monitoring). Metabolic data was analyzed using CLAX support software (Columbus Instruments).

Body composition analysis

[0165] Body composition was measured using either Dual Energy X-ray Absorptiometry (DEXA) or Echo Magnetic Resonance Imaging (EchoMRI; Echo Medical System). For DEXA, mice anesthetized using isoflurane were measured for bone mineral density and tissue composition (fat mass and lean mass) using the Lunar PIXImus. EchoMRI (Echo Medical System) was used to measure whole body composition parameters such as total body fat, lean mass, body fluids, and total body water in live mice without the need for anesthesia or sedation.

Stereotaxic injections

[0166] Four week-old *Sim1*$^{+/-}$ males or females, weighing between 22 and 26 g, were housed individually in cages for at least 2 days before surgical interventions. Mice were anesthetized with a 100 mg/kg Avertin intraperitoneal injection. The skull was immobilized in a stereotaxic apparatus (David Kopf Instruments). The stereotaxic coordinates for injection into the PVN were 0.80 mm caudal to bregma, 0 mm at the midline, and 5.2 mm below the surface of the skull. A 1.5 mm hole was created in the cranium by circular movements using hand-held Dumont 5-45 tweezers (Fine Science Tools). Using a 31 gauge 1ul Hamilton microsyringe, we injected a dose of $0.5X10^7$ vg/ml of sgRNA-AAV along with $2.5X10^6$ vg/kg of dCas-VP64-AAV, in a total injection volume of 1ul per animal into the PVN unilaterally over a 10 minute period. After AAV delivery, the needle was left in place for 20 minutes to prevent reflux and slowly withdrawn in several steps, over 10 minutes. Mice were administered two doses of buprenorphine (100mg/kg) before and 24 hours post surgery. Immunostaining for mCherry, as described below, was used to validate PVN injection coordinates 2-12 weeks following injection in several mice. Mice were maintained on a picodiet 5058 and weighed on a weekly basis.

Immunostaining

[0167] For immunostaining, mice were anesthetized with pentobarbital (7.5 mg/0.15 ml, i.p.) and transcardially perfused with 10ml of heparinized saline (10 U/ml, 2 ml/min) followed by 10ml of phosphate-buffered 4% paraformaldehyde (PFA). Brains were removed, postfixed for 24 hours in 4% PFA, and then equilibrated in 30% sucrose in PBS for 72 hours. Brains were coronally sectioned (35microns for immunostaining, 50m for stereology) on a sliding microtome (Leica SM 2000R). Immunohistochemistry was performed as previously described (19, 42, 43). Coronal brain sections that had been stored in PBS at 4°C were permeabilized and blocked in 3% normal goat serum/0.3% Triton X-100 for 1 hour and incubated at 4°C overnight using an mCherry antibody at a dilution of 1:500 (Abcam ab167453). Sections were placed in 4,6-diamidino-2-phenylindole (DAPI) (0.2 g /ml; 236276; Roche) for 10 minutes and then mounted on plus coated slides and coverslipped using Vectashield (H-1000; Vector Laboratories). Images of sections containing PVN were captured on a Zeiss Apotome.

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* * *

[0003] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, one of skill in the art will appreciate that certain changes and modifications may be practiced within the scope of the appended claims. All patents, patent applications, and other publications, including GenBank Accession Numbers, Entrez Gene IDs, and publications referred to by pubmed ID (PMID), cited in this application are incorporated by reference in the entirety for all purposes.

[00169] Where any or all of the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification (including the claims) they are to be interpreted as specifying the

presence of the stated features, integers, steps or components, but not precluding the presence of one or more other features, integers, steps or components.

[00170] A reference herein to a patent document or any other matter identified as prior art, is not to be taken as an admission that the document or other matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of treating a haploinsufficiency disease in a mammalian subject, the method comprising contacting a cell of the subject with a composition comprising:

i) a catalytically inactive CRISPR nuclease fused to a transcriptional activation domain, and

ii) a guide RNA, wherein the guide RNA comprises:

a) a targeting region that, under conditions present in a nucleus of the cell, specifically hybridizes to a promoter region or an enhancer region operably linked to a wild-type copy of a haploinsufficient gene; and

b) a binding region that specifically binds the catalytically inactive CRISPR nuclease under conditions present in a nucleus of the cell; and

-wherein the contacting forms a complex comprising the catalytically inactive CRISPR nuclease bound to the guide RNA, wherein the targeting region of the guide RNA in the complex is hybridized to the promoter or enhancer of the wild-type copy of the haploinsufficient gene, and

-wherein the complex activates transcription of the wild-type copy of the haploinsufficient gene in an amount and for a duration sufficient to treat the haploinsufficiency disease in the subject.

2. The method of claim 1, wherein the contacting comprises:

(a) contacting the cell with an episomal vector encoding the guide RNA or the catalytically inactive CRISPR nuclease; or

(b) contacting the cell with an episomal vector encoding the guide RNA and the catalytically inactive CRISPR nuclease; or.

(c) contacting the cell with an episomal vector encoding the guide RNA and a second episomal vector encoding the catalytically inactive CRISPR nuclease; or

(d) injection of nucleic acid encoding the guide RNA and/or the catalytically inactive CRISPR nuclease into a region of a brain containing a hypothalamus; or

(e) injection of an adeno-associated viral vector comprising a nucleic acid encoding the guide RNA and/or the catalytically inactive CRISPR nuclease into a region of a brain containing a hypothalamus.

3. The method of claim 2, wherein the episomal vector(s):

(a) are non-integrating; and/or

(b) are non-replicating; and/or

(c) are adeno-associated virus (AAV) vectors; and/or

(d) independently comprise a first and a second end, wherein the first end and second end each independently comprise an AAV inverted terminal repeat.

4. The method of any one of claims 1 to 3, wherein the catalytically inactive CRISPR nuclease comprises (i) a nuclease domain that has been modified to eliminate nuclease and nicking activity and (ii) a transcriptional activation domain and/or D10A, H840 S. *pyogenes* Cas9.

5. The method of any one of claims 1 to 4, wherein the catalytically inactive CRISPR nuclease is a CRISPR nuclease-VP64 fusion polypeptide.

6. The method of any one of claims 1 to 5, wherein the haploinsufficient gene is *SIM1*, *MC4R, SCN2A, or SCN1A*.

7. The method of any one of claims 1 to 6, wherein the cell is a non-dividing cell, a neuron., or a hypothalamus cell.

8. The method of any one of claims 1 to 7, wherein the haploinsufficiency disease is selected from the group consisting of obesity, autism, epilepsy, intellectual disability, aniridia, and polycystic kidney disease.

9. Use of a composition in the manufacture of a medicament for the treatment of a haploinsufficiency disease in a mammalian subject, the composition comprising:

i) a catalytically inactive CRISPR nuclease fused to a transcriptional activation domain, and

ii) a guide RNA, wherein the guide RNA comprises:

a) a targeting region that, under conditions present in a nucleus of the cell, specifically hybridizes to a promoter region or an enhancer region operably linked to a wild-type copy of a haploinsufficient gene; and

b) a binding region that specifically binds the catalytically inactive CRISPR nuclease under conditions present in a nucleus of the cell; and

-wherein the medicament is adapted to make the composition contact with a cell of the subject to form a complex comprising the CRISPR nuclease bound to the guide RNA, wherein the targeting region of the guide RNA in the complex is hybridized to the promoter or enhancer of the wild-type copy of the haploinsufficient gene, and

-wherein the complex activates transcription of the wild-type copy of the haploinsufficient gene in an amount and for a duration sufficient to treat the haploinsufficiency disease in the subject.

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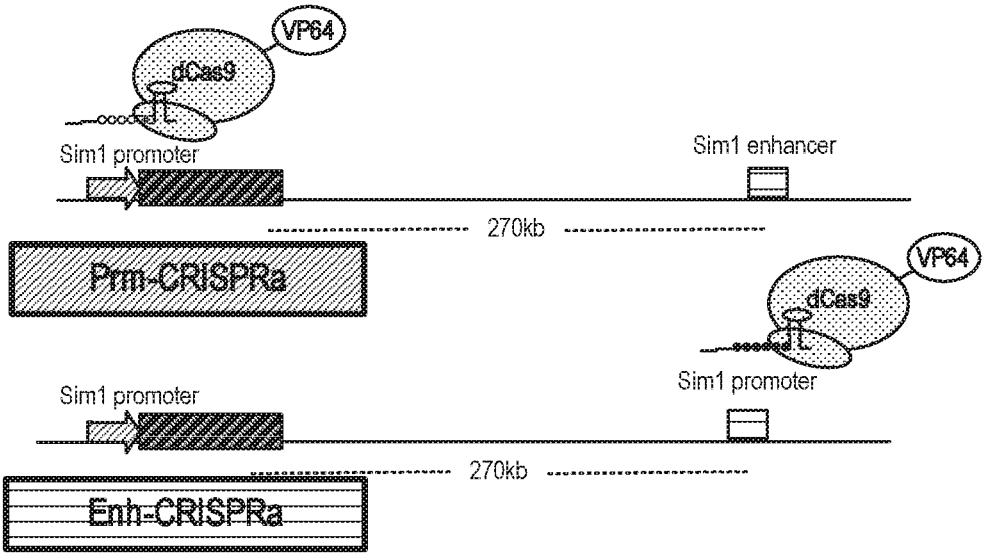

FIG. 1a

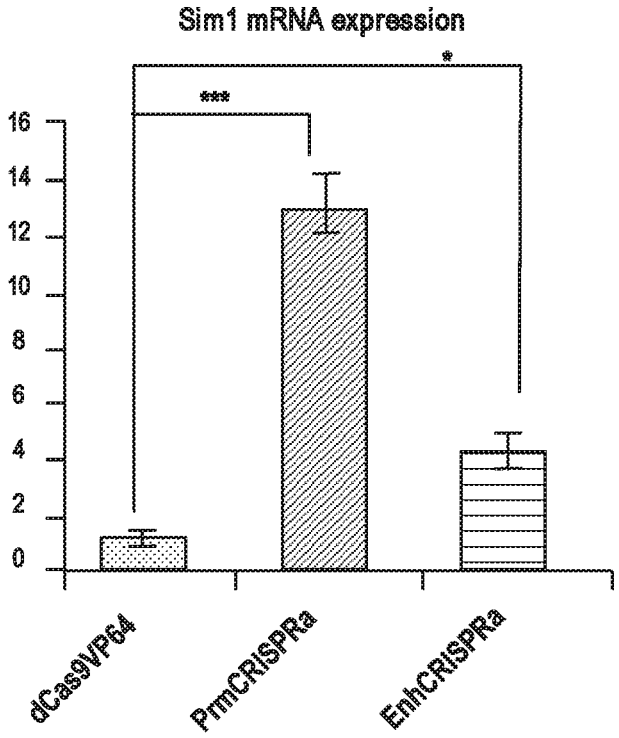

FIG. 1b

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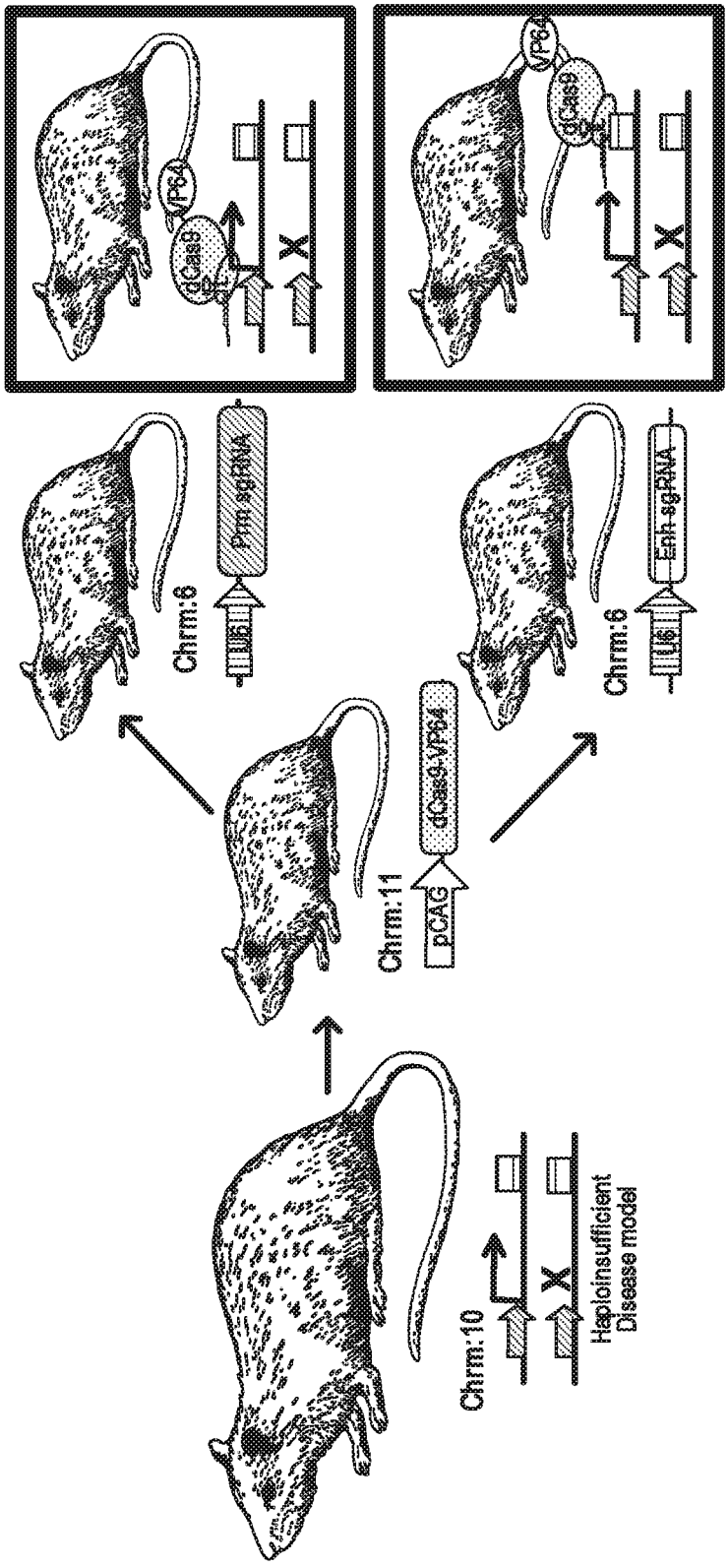

FIG. 1c

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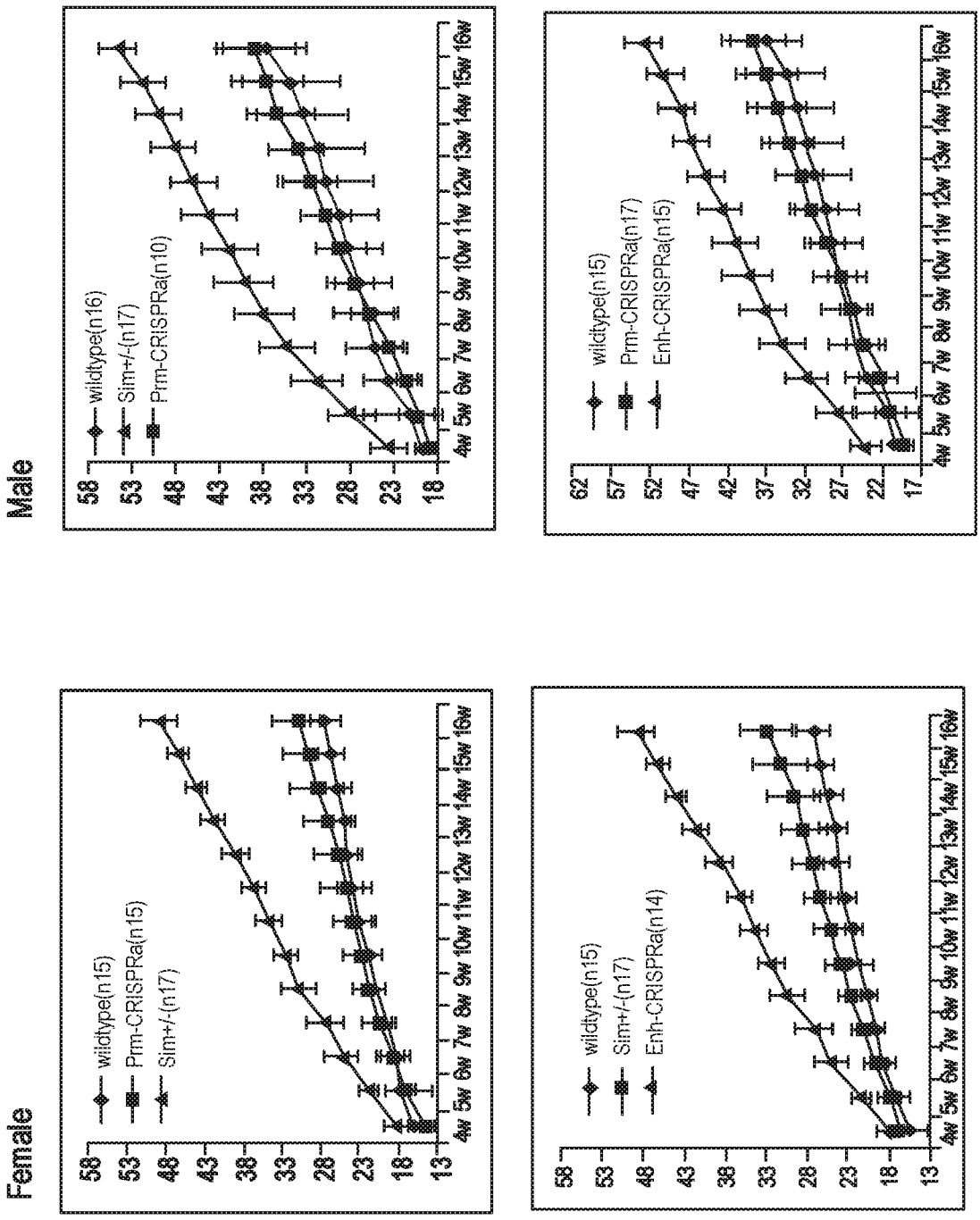

FIG. 1d

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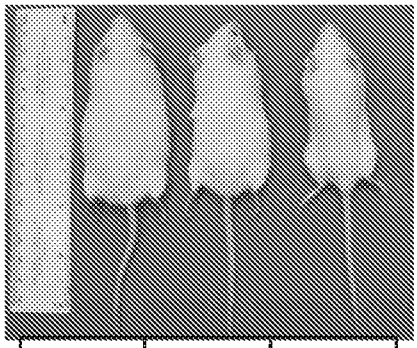
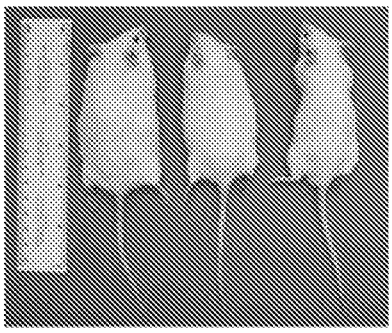
Sim1+/- Prm-CRISPa Wildtype



10cm	9.5cm	9cm	Length
43gms	31gms	24gm	Weight

FIG. 1e

Sim1+/- Enh-CRISPa Wildtype



11cm	10cm	10cm	Length
41gm	28gms	25gms	Weight

FIG. 1f

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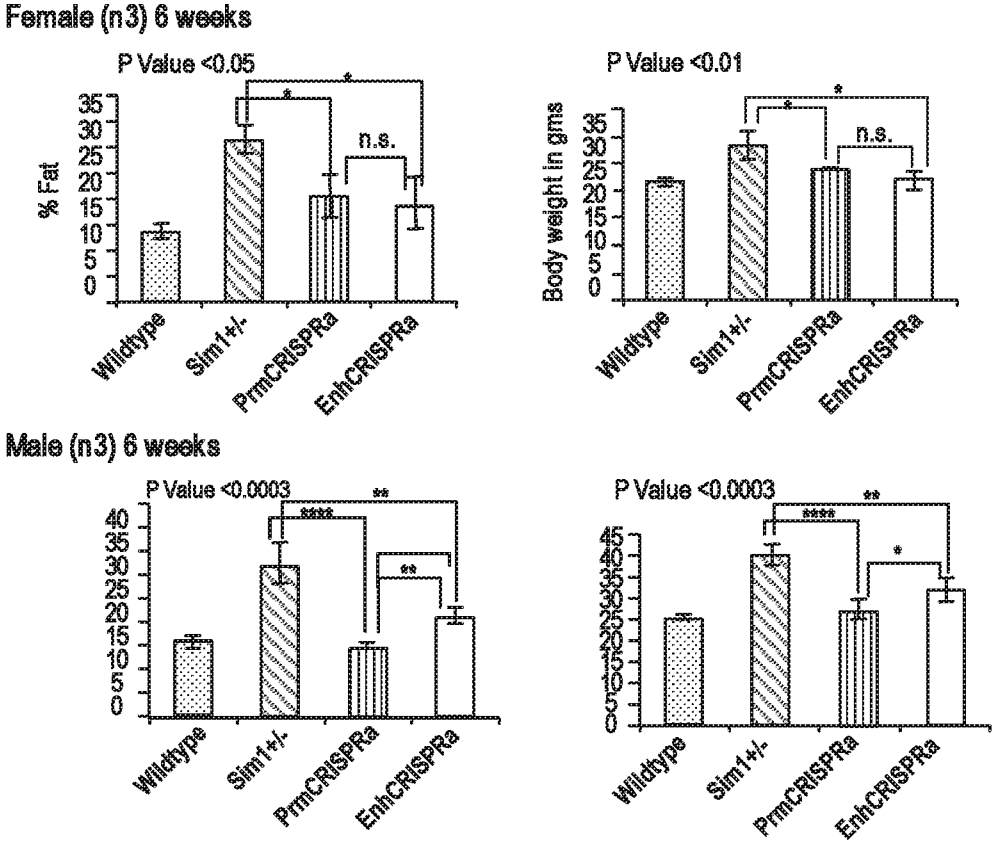

FIG. 2a

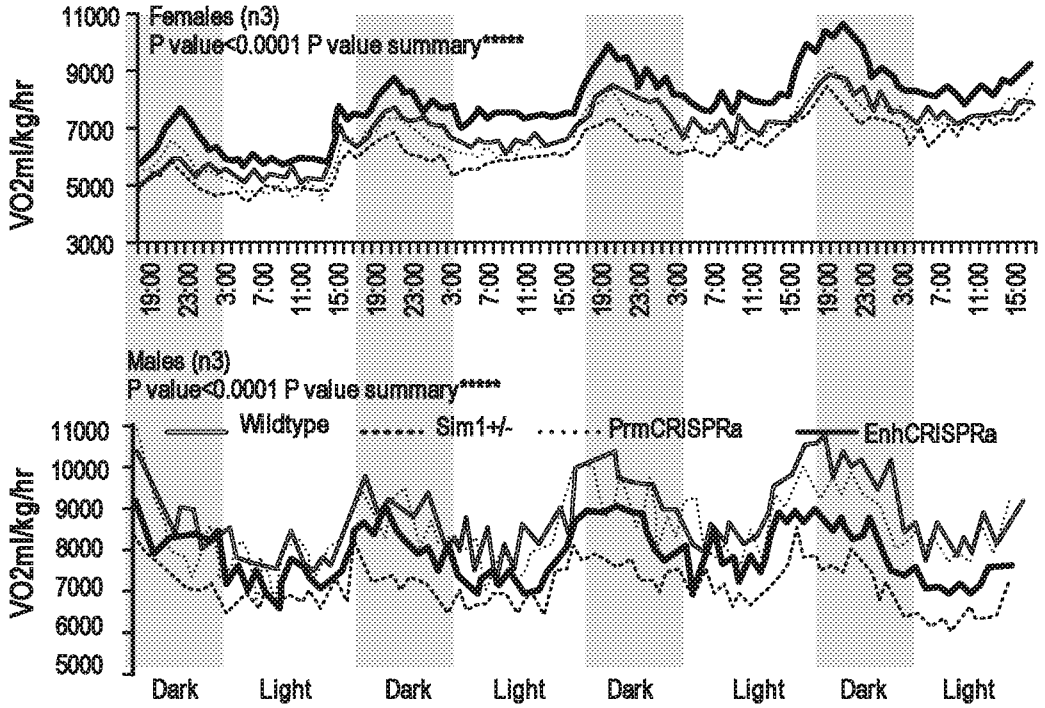

FIG. 2b

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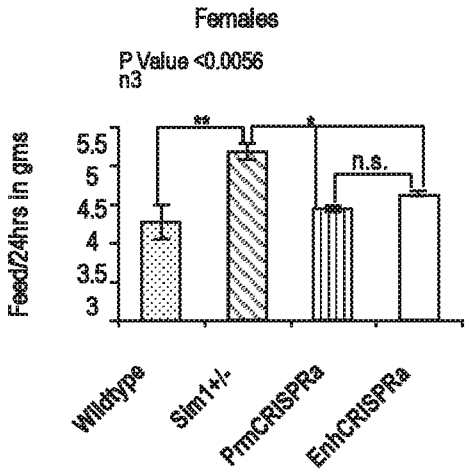

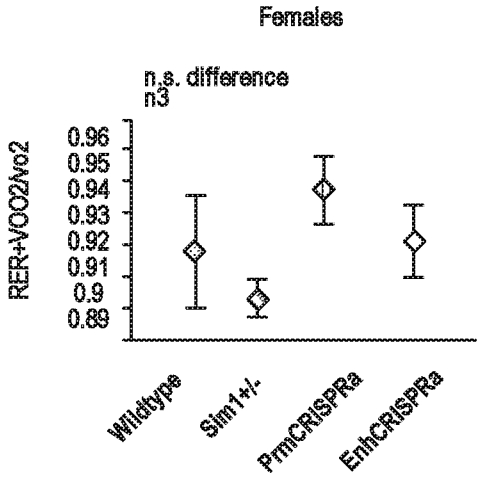

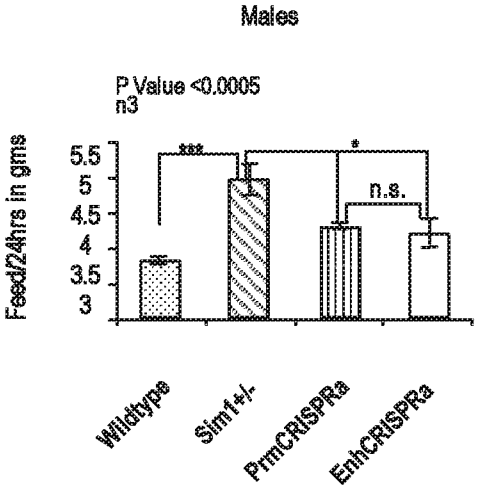

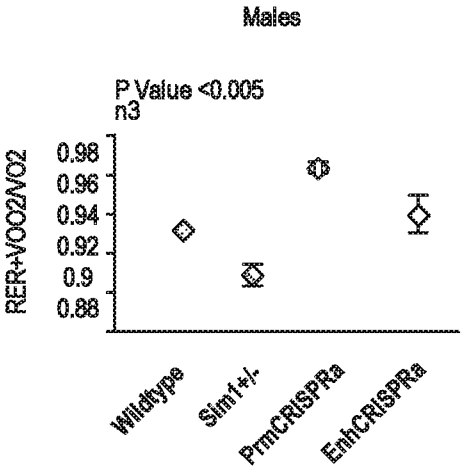

FIG. 2c

FIG. 2d

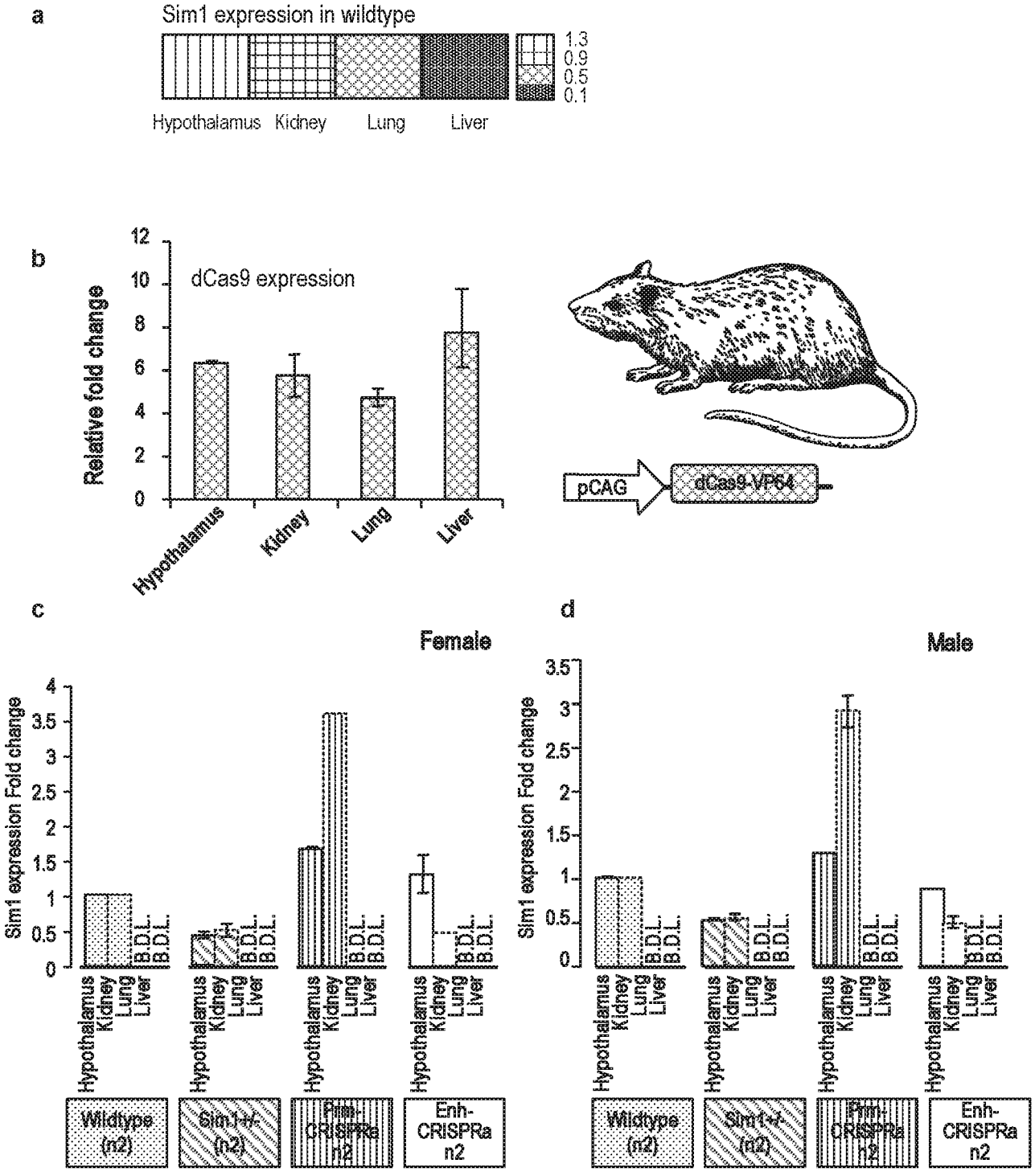

FIG. 3

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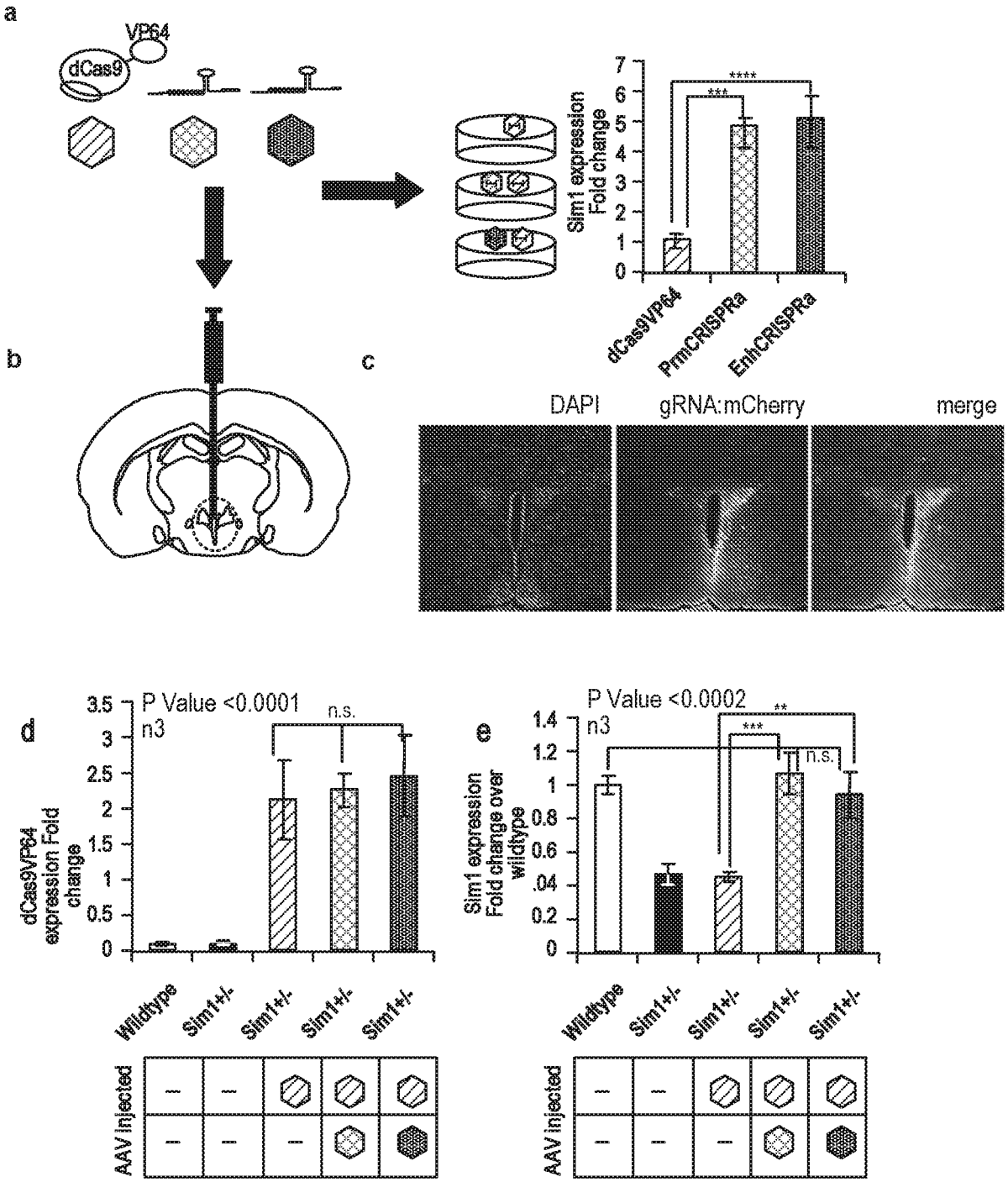

FIG. 4

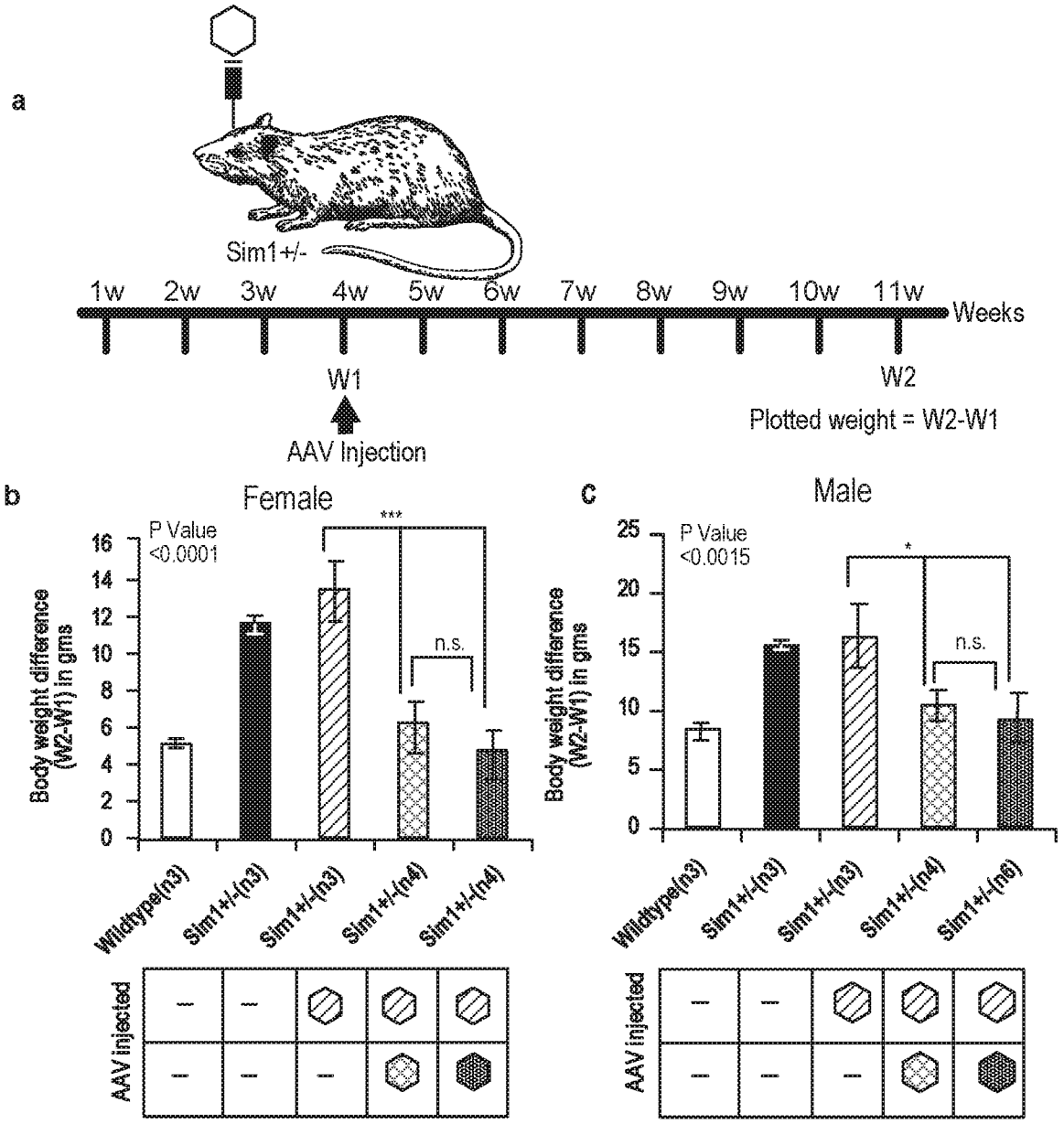

FIG. 5

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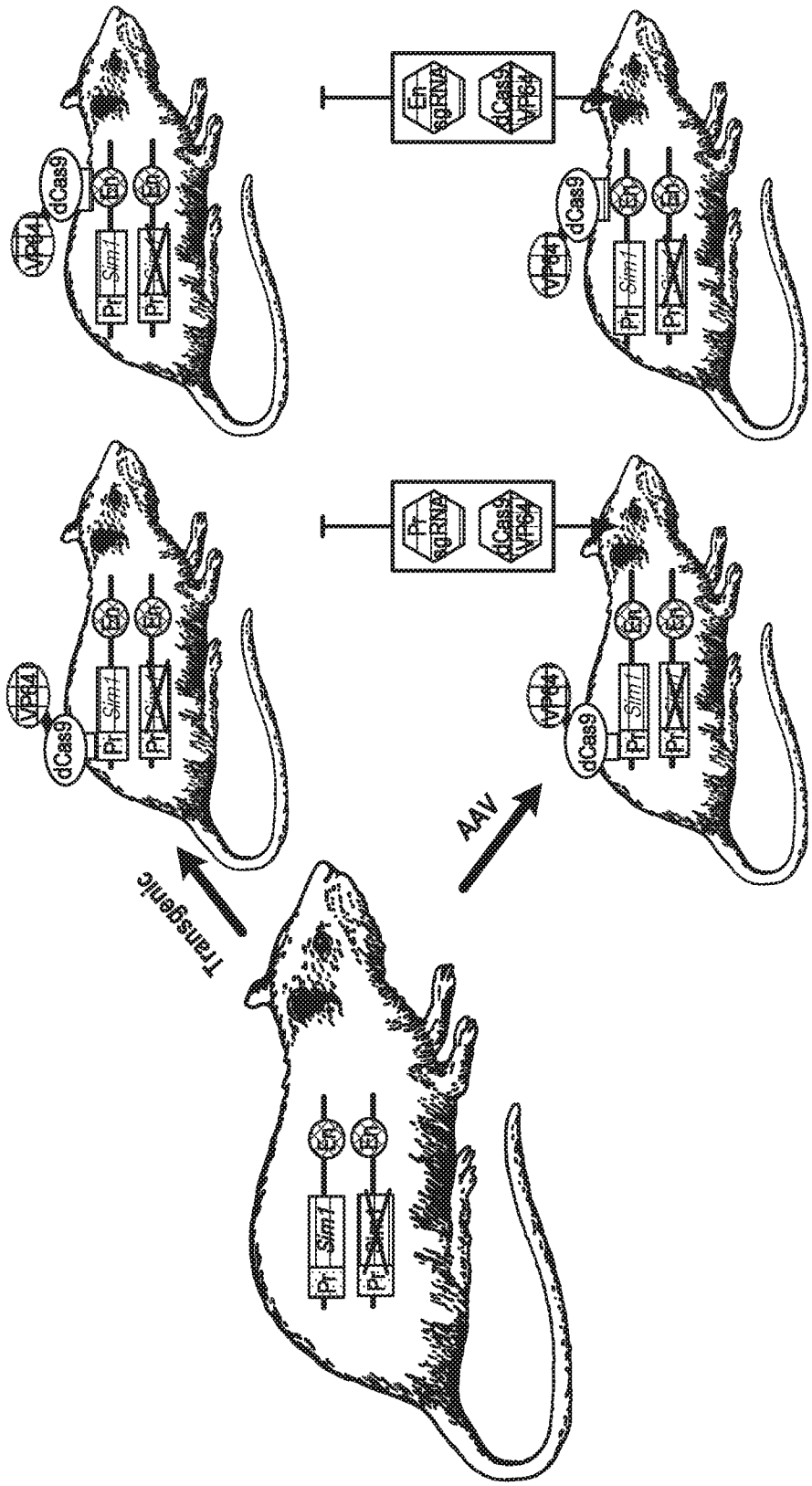

FIG. 6

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Fig. 7A

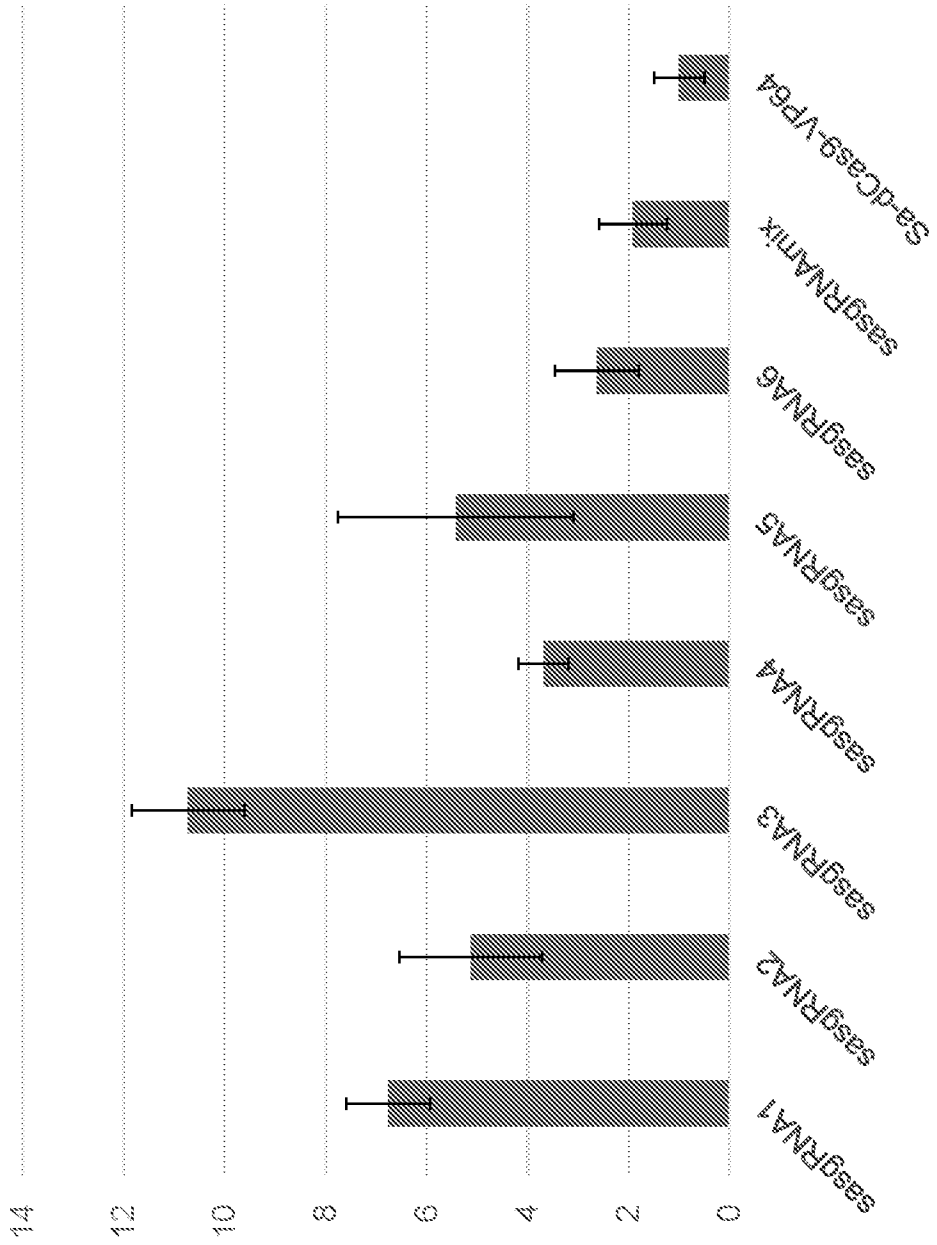

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Fig. 7B

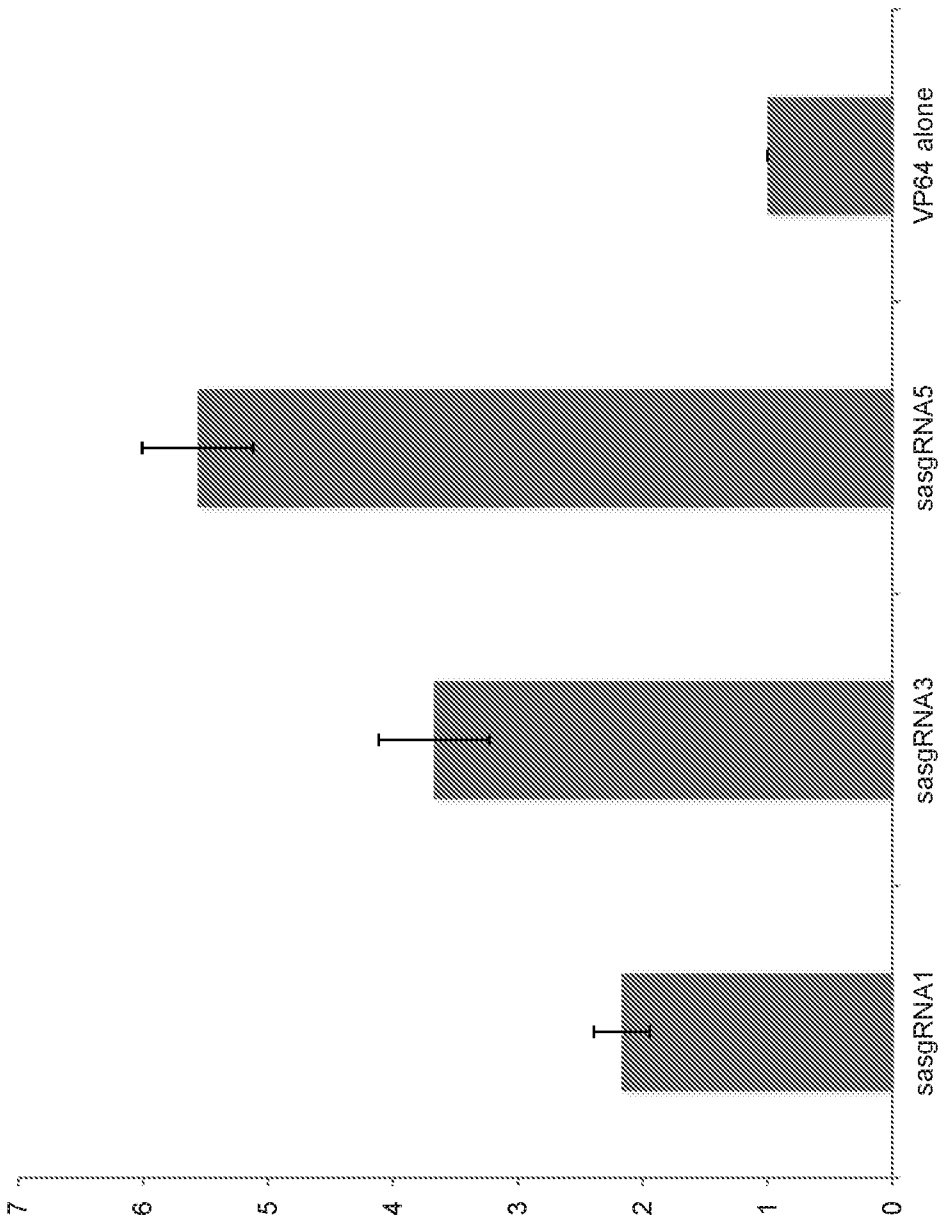

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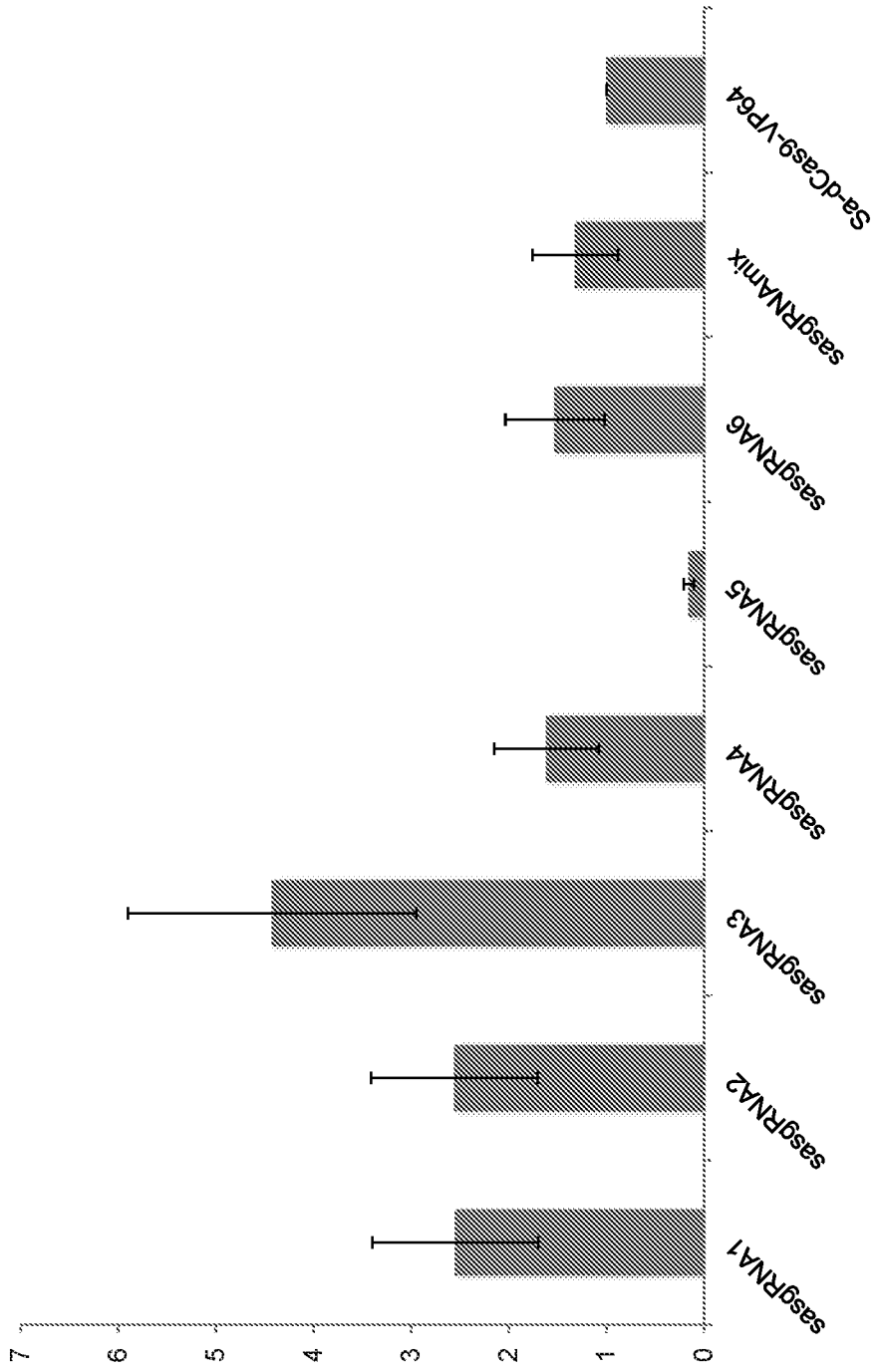
Fig. 8A



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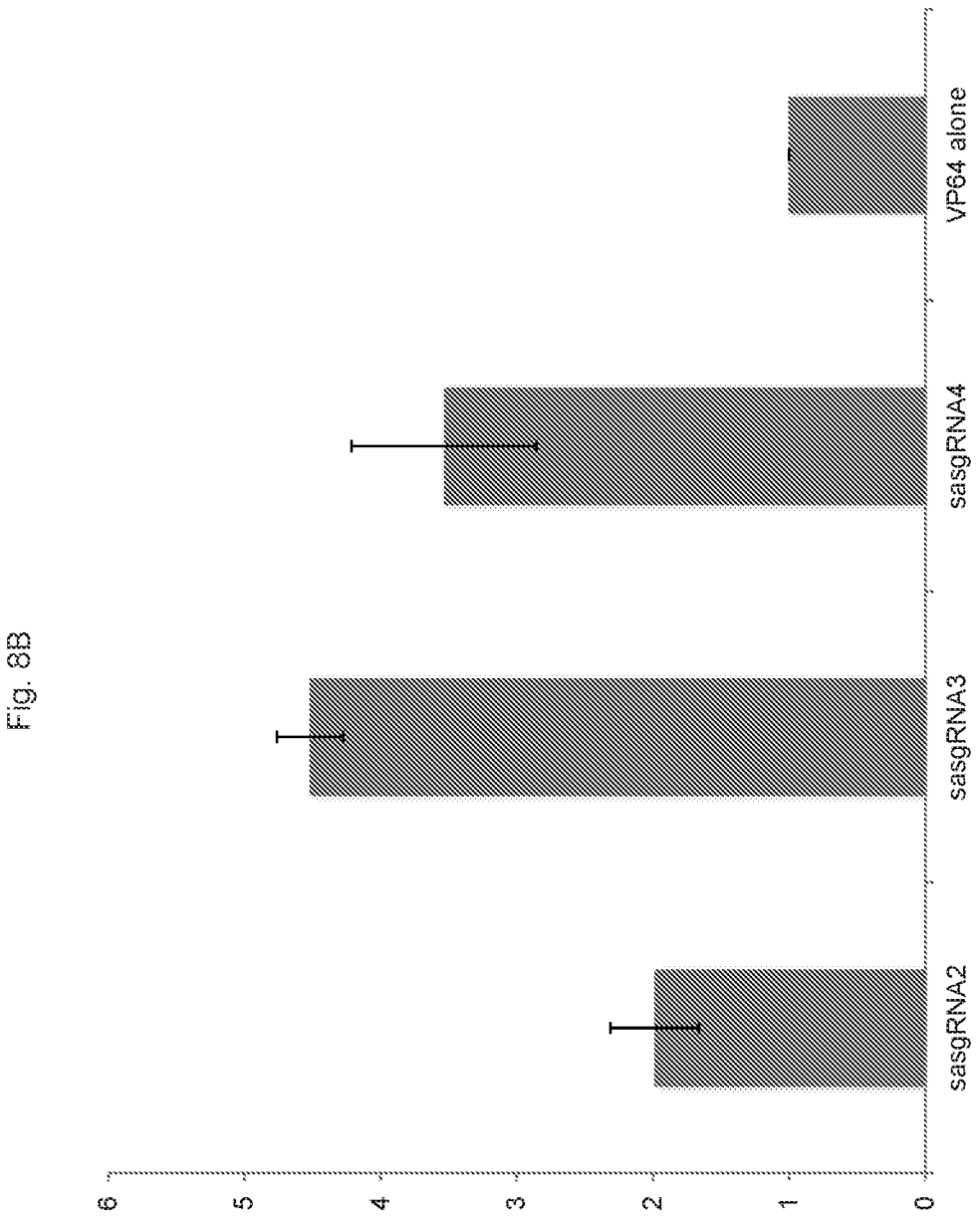

Fig. 8B

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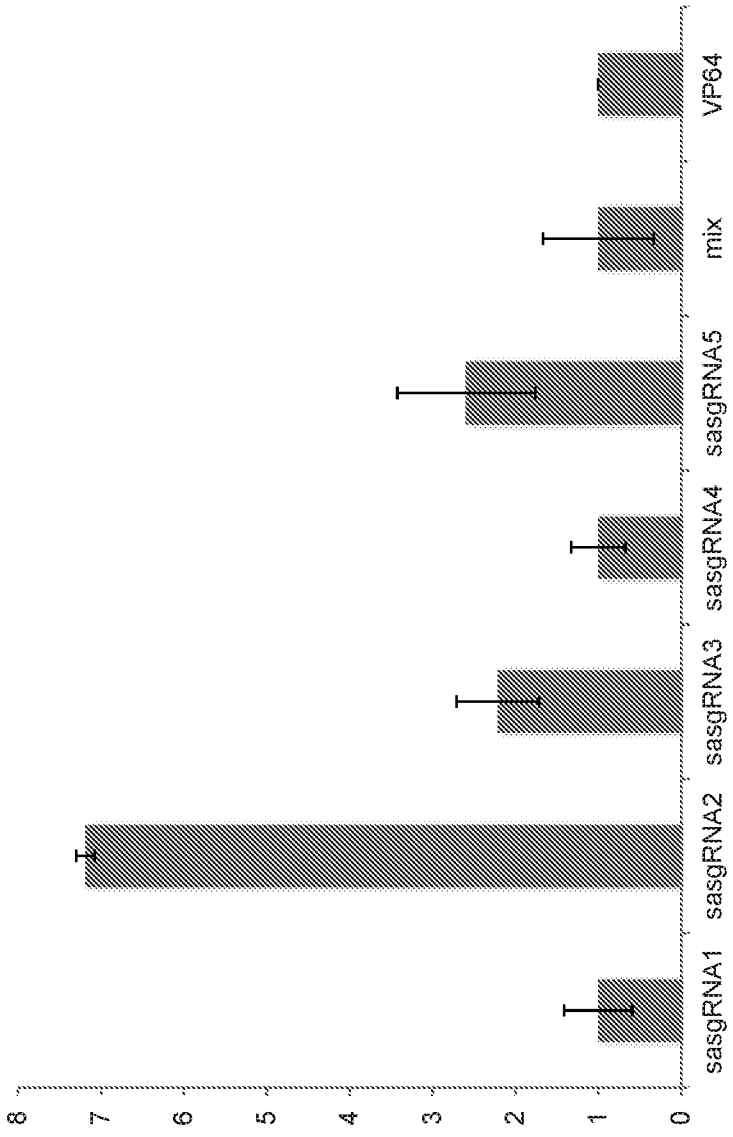
Fig. 9A



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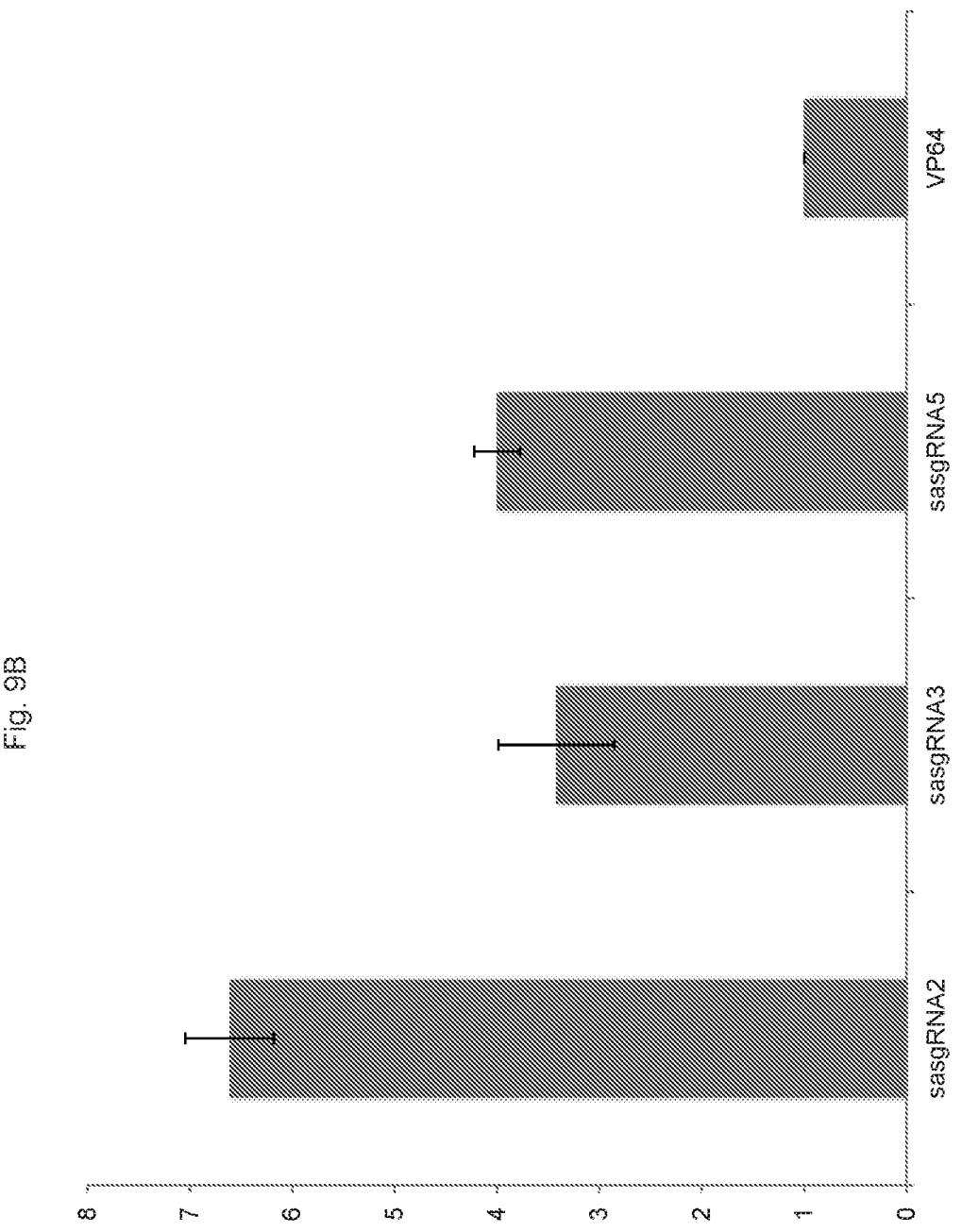

Fig. 9B

2018218280 17 Oct 2019

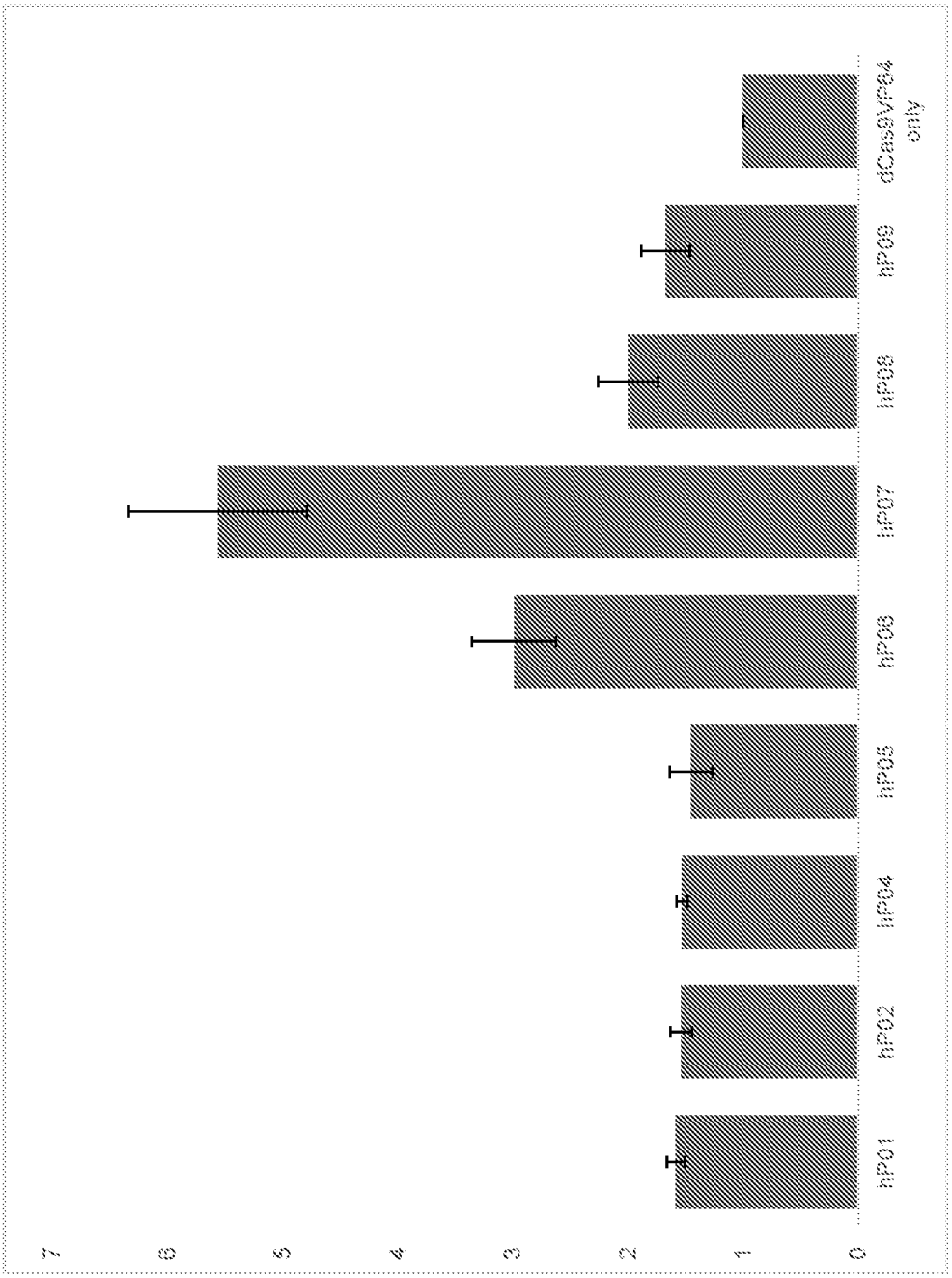

Fig. 10 PDK1

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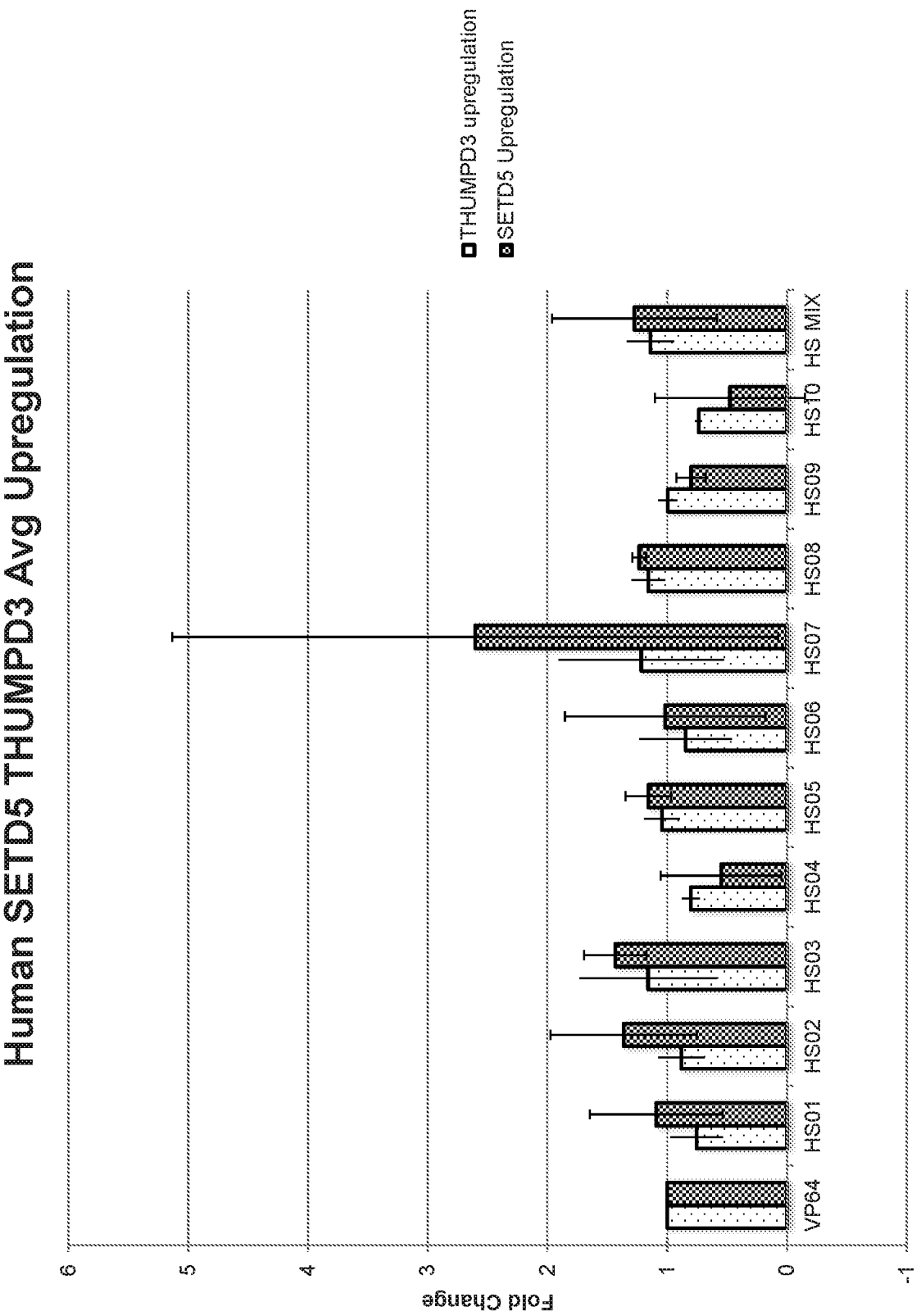
Fig. 11A



2018218280 17 Oct 2019

Fig. 11B

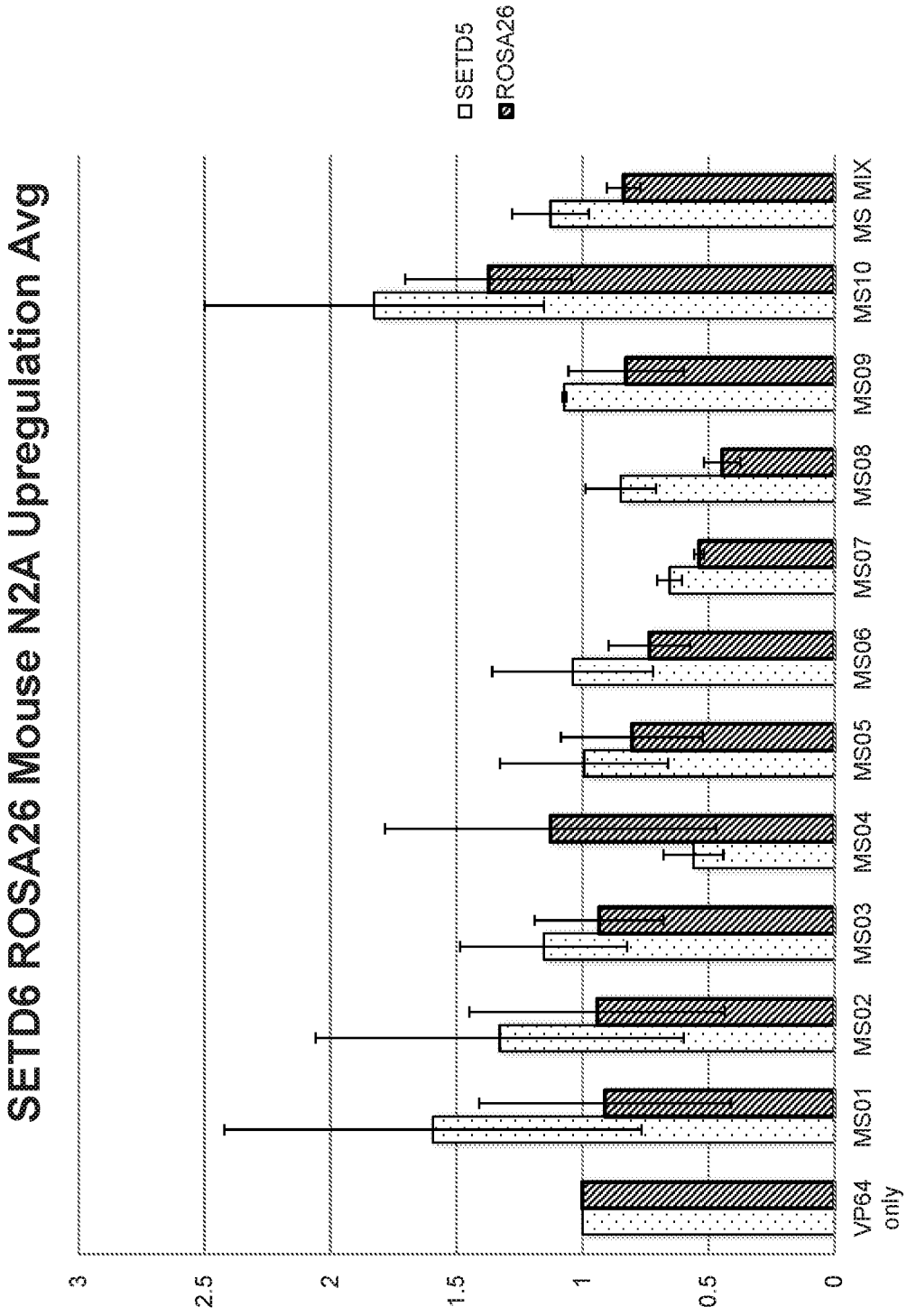


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Fig. 12A

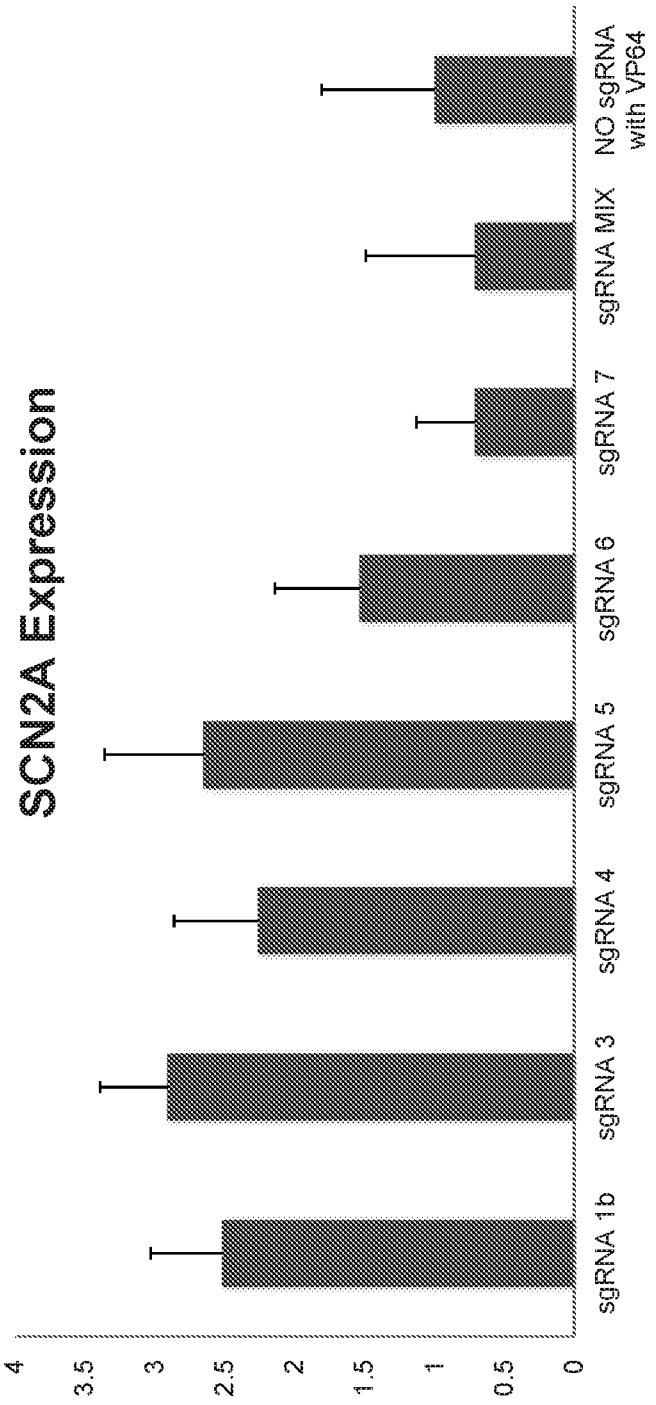

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Fig. 12B

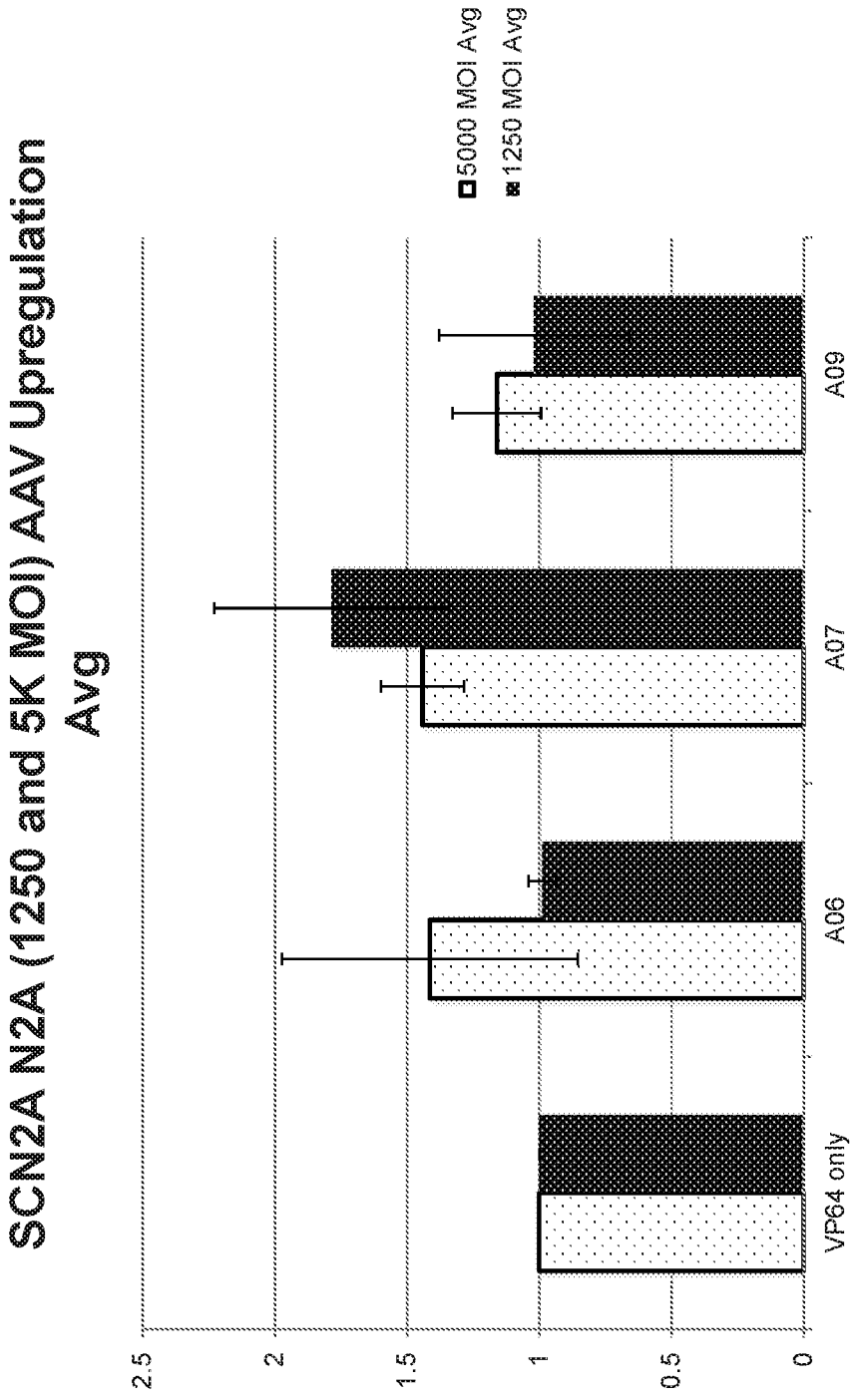

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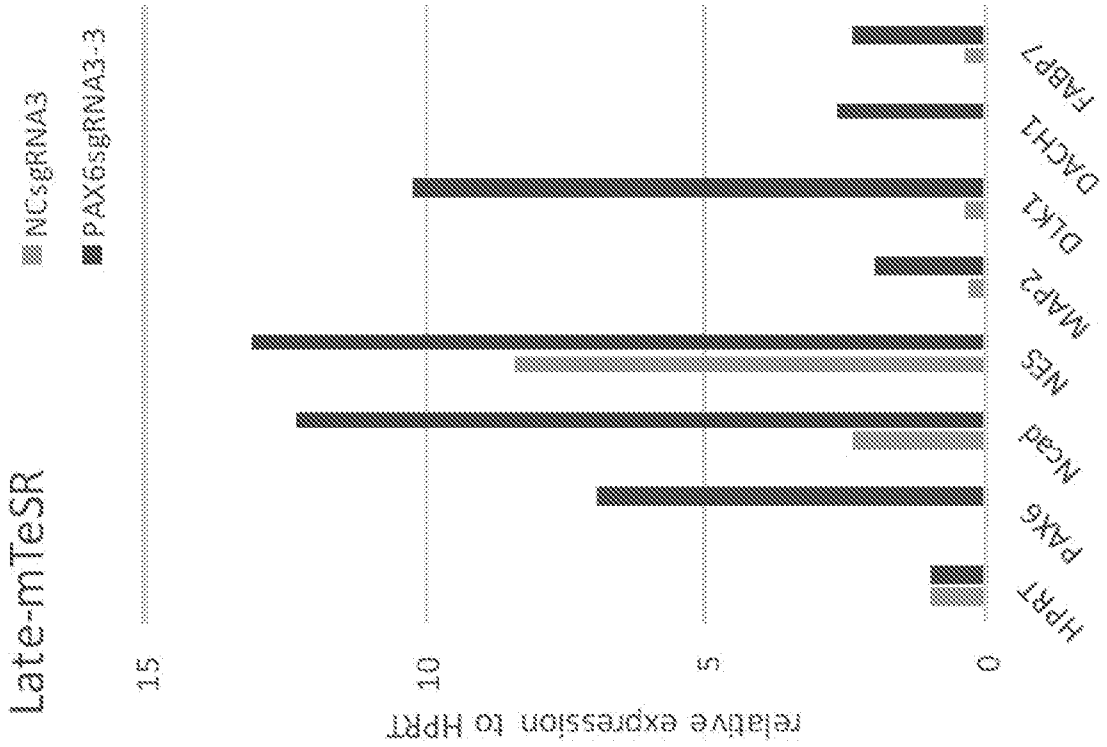
Fig. 13



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His Leu Asp Ala Met Asp Ser Asn Leu Asp Asn Leu Gln Thr Met Leu
20 25 30

Ser Ser His Gly Phe Ser Val Asp Thr Ser Ala Leu Leu Asp Leu Phe
35 40 45

Ser Pro Ser Val Thr Val Pro Asp Met Ser Leu Pro Asp Leu Asp Ser
50 55 60

Ser Leu Ala Ser Ile Gln Glu Leu Leu Ser Pro Gln Glu Pro Pro Arg
65 70 75 80

Pro Pro Glu Ala Glu Asn Ser Ser Pro Asp Ser Gly Lys Gln Leu Val
85 90 95

His Tyr Thr Ala Gln Pro Leu Phe Leu Leu Asp Pro Gly Ser Val Asp
100 105 110

```
Thr Gly Ser Asn Asp Leu Pro Val Leu Phe Glu Leu Gly Glu Gly Ser
        115                 120                 125


Tyr Phe Ser Glu Gly Asp Gly Phe Ala Glu Asp Pro Thr Ile Ser Leu
    130                 135                 140


Leu Thr Gly Ser Glu Pro Pro Lys Ala Lys Asp Pro Thr Val Ser
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Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe
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Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu Asp
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Met Leu
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<213> Homo sapiens

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Arg Lys Arg Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys Lys Ser Pro
20 25 30

Phe Ser Gly Pro Thr Asp Pro Arg Pro Pro Pro Arg Arg Ile Ala Val
35 40 45

Pro Ser Arg Ser Ser Ala Ser Val Pro Lys Pro Ala Pro Gln Pro Tyr
50 55 60

Pro Phe Thr Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu Phe Pro Thr
65 70 75 80

Met Val Phe Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala Leu Ala Pro
85 90 95

Ala Pro Pro Gln Val Leu Pro Gln Ala Pro Ala Pro Ala Pro Ala Pro
100 105 110

Ala Met Val Ser Ala Leu Ala Gln Ala Pro Ala Pro Val Pro Val Leu
115 120 125

Ala Pro Gly Pro Pro Gln Ala Val Ala Pro Pro Ala Pro Lys Pro Thr
130 135 140

```
Gln Ala Gly Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln Leu Gln Phe
145                 150                 155                 160


Asp Asp Glu Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr Asp Pro Ala
                165                 170                 175


Val Phe Thr Asp Leu Ala Ser Val Asp Asn Ser Glu Phe Gln Gln Leu
            180                 185                 190


Leu Asn Gln Gly Ile Pro Val Ala Pro His Thr Thr Glu Pro Met Leu
        195                 200                 205


Met Glu Tyr Pro Glu Ala Ile Thr Arg Leu Val Thr Gly Ala Gln Arg
    210                 215                 220


Pro Pro Asp Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly Leu Pro Asn
225                 230                 235                 240


Gly Leu Leu Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala Asp Met Asp
                245                 250                 255


Phe Ser Ala Leu Leu
            260


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<212>  PRT
<213>  Mus musculus

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Met Glu Leu Leu Ser Pro Pro Leu Arg Asp Ile Asp Leu Thr Gly Pro
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Asp Gly Ser Leu Cys Ser Phe Glu Thr Ala Asp Asp Phe Tyr Asp Asp
            20                  25                  30
```

```
Pro Cys Phe Asp Ser Pro Asp Leu Arg Phe Phe Glu Asp Leu Asp Pro
        35                  40                  45

Arg Leu Val His Met Gly Ala Leu Leu Lys Pro Glu Glu His Ala His
    50                  55                  60

Phe Pro Thr Ala Val His Pro Gly Pro Gly Ala Arg Glu Asp Glu His
65                  70                  75                  80

Val Arg Ala Pro Ser Gly His His Gln Ala Gly Arg Cys Leu Leu Trp
                85                  90                  95

Ala Cys Lys Ala Cys Lys Arg Lys Thr Thr Asn Ala Asp Arg Arg Lys
            100                 105                 110

Ala Ala Thr Met Arg Glu Arg Arg Arg Leu Ser Lys Val Asn Glu Ala
        115                 120                 125

Phe Glu Thr Leu Lys Arg Cys Thr Ser Ser Asn Pro Asn Gln Arg Leu
    130                 135                 140

Pro Lys Val Glu Ile Leu Arg Asn Ala Ile Arg Tyr Ile Glu Gly Leu
145                 150                 155                 160

Gln Ala Leu Leu Arg Asp Gln Asp Ala Ala Pro Pro Gly Ala Ala Ala
                165                 170                 175

Phe Tyr Ala Pro Gly Pro Leu Pro Pro Gly Arg Gly Ser Glu His Tyr
            180                 185                 190

Ser Gly Asp Ser Asp Ala Ser Ser Pro Arg Ser Asn Cys Ser Asp Gly
        195                 200                 205

Met Met Asp Tyr Ser Gly Pro Pro Ser Gly Pro Arg Arg Gln Asn Gly
    210                 215                 220
```

```
Tyr Asp Thr Ala Tyr Tyr Ser Glu Ala Ala Arg Glu Ser Arg Pro Gly
225                 230                 235                 240


Lys Ser Ala Ala Val Ser Ser Leu Asp Cys Leu Ser Ser Ile Val Glu
                245                 250                 255


Arg Ile Ser Thr Asp Ser Pro Ala Ala Pro Ala Leu Leu Leu Ala Asp
            260                 265                 270


Ala Pro Pro Glu Ser Pro Pro Gly Pro Pro Glu Gly Ala Ser Leu Ser
        275                 280                 285


Asp Thr Glu Gln Gly Thr Gln Thr Pro Ser Pro Asp Ala Ala Pro Gln
    290                 295                 300


Cys Pro Ala Gly Ser Asn Pro Asn Ala Ile Tyr Gln Val Leu
305                 310                 315


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<213>  Unknown

<220>
<223>  Epstein-Barr virus

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Arg Asp Ser Arg Glu Gly Met Phe Leu Pro Lys Pro Glu Ala Gly Ser
1               5                   10                  15


Ala Ile Ser Asp Val Phe Glu Gly Arg Glu Val Cys Gln Pro Lys Arg
            20                  25                  30


Ile Arg Pro Phe His Pro Pro Gly Ser Pro Trp Ala Asn Arg Pro Leu
        35                  40                  45


Pro Ala Ser Leu Ala Pro Thr Pro Thr Gly Pro Val His Glu Pro Val
    50                  55                  60
```

```
Gly Ser Leu Thr Pro Ala Pro Val Pro Gln Pro Leu Asp Pro Ala Pro
65                  70                  75                  80


Ala Val Thr Pro Glu Ala Ser His Leu Leu Glu Asp Pro Asp Glu Glu
                85                  90                  95


Thr Ser Gln Ala Val Lys Ala Leu Arg Glu Met Ala Asp Thr Val Ile
            100                 105                 110


Pro Gln Lys Glu Glu Ala Ala Ile Cys Gly Gln Met Asp Leu Ser His
        115                 120                 125


Pro Pro Pro Arg Gly His Leu Asp Glu Leu Thr Thr Thr Leu Glu Ser
    130                 135                 140


Met Thr Glu Asp Leu Asn Leu Asp Ser Pro Leu Thr Pro Glu Leu Asn
145                 150                 155                 160


Glu Ile Leu Asp Thr Phe Leu Asn Asp Glu Cys Leu Leu His Ala Met
                165                 170                 175


His Ile Ser Thr Gly Leu Ser Ile Phe Asp Thr Ser Leu Phe
            180                 185                 190


<210>  19
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Met Asp Ser Asp Asp Glu Met Val Glu Glu Ala Val Glu Gly His Leu
1               5                   10                  15


Asp Asp Asp Gly Leu Pro His Gly Phe Cys Thr Val Thr Tyr Ser Ser
            20                  25                  30
```

```
Thr Asp Arg Phe Glu Gly Asn Phe Val His Gly Glu Lys Asn Gly Arg
        35                  40                  45

Gly Lys Phe Phe Phe Phe Asp Gly Ser Thr Leu Glu Gly Tyr Tyr Val
    50                  55                  60

Asp Asp Ala Leu Gln Gly Gln Gly Val Tyr Thr Tyr Glu Asp Gly Gly
65                  70                  75                  80

Val Leu Gln Gly Thr Tyr Val Asp Gly Glu Leu Asn Gly Pro Ala Gln
                85                  90                  95

Glu Tyr Asp Thr Asp Gly Arg Leu Ile Phe Lys Gly Gln Tyr Lys Asp
            100                 105                 110

Asn Ile Arg His Gly Val Cys Trp Ile Tyr Tyr Pro Asp Gly Gly Ser
        115                 120                 125

Leu Val Gly Glu Val Asn Glu Asp Gly Glu Met Thr Gly Glu Lys Ile
    130                 135                 140

Ala Tyr Val Tyr Pro Asp Glu Arg Thr Ala Leu Tyr Gly Lys Phe Ile
145                 150                 155                 160

Asp Gly Glu Met Ile Glu Gly Lys Leu Ala Thr Leu Met Ser Thr Glu
                165                 170                 175

Glu Gly Arg Pro His Phe Glu Leu Met Pro Gly Asn Ser Val Tyr His
            180                 185                 190

Phe Asp Lys Ser Thr Ser Ser Cys Ile Ser Thr Asn Ala Leu Leu Pro
        195                 200                 205

Asp Pro Tyr Glu Ser Glu Arg Val Tyr Val Ala Glu Ser Leu Ile Ser
    210                 215                 220
```

```
Ser Ala Gly Glu Gly Leu Phe Ser Lys Val Ala Val Gly Pro Asn Thr
225                 230                 235                 240


Val Met Ser Phe Tyr Asn Gly Val Arg Ile Thr His Gln Glu Val Asp
                245                 250                 255


Ser Arg Asp Trp Ala Leu Asn Gly Asn Thr Leu Ser Leu Asp Glu Glu
            260                 265                 270


Thr Val Ile Asp Val Pro Glu Pro Tyr Asn His Val Ser Lys Tyr Cys
        275                 280                 285


Ala Ser Leu Gly His Lys Ala Asn His Ser Phe Thr Pro Asn Cys Ile
    290                 295                 300


Tyr Asp Met Phe Val His Pro Arg Phe Gly Pro Ile Lys Cys Ile Arg
305                 310                 315                 320


Thr Leu Arg Ala Val Glu Ala Asp Glu Glu Leu Thr Val Ala Tyr Gly
                325                 330                 335


Tyr Asp His Ser Pro Pro Gly Lys Ser Gly Pro Glu Ala Pro Glu Trp
            340                 345                 350


Tyr Gln Val Glu Leu Lys Ala Phe Gln Ala Thr Gln Gln Lys
        355                 360                 365


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Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu
            20                  25                  30

Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp
        35                  40                  45

Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Ile Asn Ser Arg Ser Ser
    50                  55                  60

Gly Ser Pro Lys Lys Lys Arg Lys Val Gly Ser Gln Tyr Leu Pro Asp
65                  70                  75                  80

Thr Asp Asp Arg His Arg Ile Glu Glu Lys Arg Lys Arg Thr Tyr Glu
                85                  90                  95

Thr Phe Lys Ser Ile Met Lys Lys Ser Pro Phe Ser Gly Pro Thr Asp
            100                 105                 110

Pro Arg Pro Pro Pro Arg Arg Ile Ala Val Pro Ser Arg Ser Ser Ala
        115                 120                 125

Ser Val Pro Lys Pro Ala Pro Gln Pro Tyr Pro Phe Thr Ser Ser Leu
    130                 135                 140

Ser Thr Ile Asn Tyr Asp Glu Phe Pro Thr Met Val Phe Pro Ser Gly
145                 150                 155                 160

Gln Ile Ser Gln Ala Ser Ala Leu Ala Pro Ala Pro Pro Gln Val Leu
                165                 170                 175

Pro Gln Ala Pro Ala Pro Ala Pro Ala Pro Ala Met Val Ser Ala Leu
            180                 185                 190

Ala Gln Ala Pro Ala Pro Val Pro Val Leu Ala Pro Gly Pro Pro Gln
```

```
        195                 200                 205

Ala Val Ala Pro Pro Ala Pro Lys Pro Thr Gln Ala Gly Glu Gly Thr
    210                 215                 220

Leu Ser Glu Ala Leu Leu Gln Leu Gln Phe Asp Asp Glu Asp Leu Gly
225                 230                 235                 240

Ala Leu Leu Gly Asn Ser Thr Asp Pro Ala Val Phe Thr Asp Leu Ala
                245                 250                 255

Ser Val Asp Asn Ser Glu Phe Gln Gln Leu Leu Asn Gln Gly Ile Pro
            260                 265                 270

Val Ala Pro His Thr Thr Glu Pro Met Leu Met Glu Tyr Pro Glu Ala
        275                 280                 285

Ile Thr Arg Leu Val Thr Gly Ala Gln Arg Pro Pro Asp Pro Ala Pro
    290                 295                 300

Ala Pro Leu Gly Ala Pro Gly Leu Pro Asn Gly Leu Leu Ser Gly Asp
305                 310                 315                 320

Glu Asp Phe Ser Ser Ile Ala Asp Met Asp Phe Ser Ala Leu Leu Gly
                325                 330                 335

Ser Gly Ser Gly Ser Arg Asp Ser Arg Glu Gly Met Phe Leu Pro Lys
            340                 345                 350

Pro Glu Ala Gly Ser Ala Ile Ser Asp Val Phe Glu Gly Arg Glu Val
        355                 360                 365

Cys Gln Pro Lys Arg Ile Arg Pro Phe His Pro Pro Gly Ser Pro Trp
    370                 375                 380

Ala Asn Arg Pro Leu Pro Ala Ser Leu Ala Pro Thr Pro Thr Gly Pro
```

```
385                 390                 395                 400

Val His Glu Pro Val Gly Ser Leu Thr Pro Ala Pro Val Pro Gln Pro
                405                 410                 415

Leu Asp Pro Ala Pro Ala Val Thr Pro Glu Ala Ser His Leu Leu Glu
            420                 425                 430

Asp Pro Asp Glu Glu Thr Ser Gln Ala Val Lys Ala Leu Arg Glu Met
        435                 440                 445

Ala Asp Thr Val Ile Pro Gln Lys Glu Glu Ala Ala Ile Cys Gly Gln
    450                 455                 460

Met Asp Leu Ser His Pro Pro Pro Arg Gly His Leu Asp Glu Leu Thr
465                 470                 475                 480

Thr Thr Leu Glu Ser Met Thr Glu Asp Leu Asn Leu Asp Ser Pro Leu
                485                 490                 495

Thr Pro Glu Leu Asn Glu Ile Leu Asp Thr Phe Leu Asn Asp Glu Cys
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Leu Leu His Ala Met His Ile Ser Thr Gly Leu Ser Ile Phe Asp Thr
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Ser Leu Phe
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<213>  Homo sapiens

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```
Glu Asn Arg Val Asn Asp Phe Leu Arg Arg Gln Asn His Pro Glu Ser
            20                  25                  30

Gly Glu Val Thr Val Arg Val Val His Ala Ser Asp Lys Thr Val Glu
        35                  40                  45

Val Lys Pro Gly Met Lys Ala Arg Phe Val Asp Ser Gly Glu Met Ala
    50                  55                  60

Glu Ser Phe Pro Tyr Arg Thr Lys Ala Leu Phe Ala Phe Glu Glu Ile
65                  70                  75                  80

Asp Gly Val Asp Leu Cys Phe Phe Gly Met His Val Gln Glu Tyr Gly
                85                  90                  95

Ser Asp Cys Pro Pro Pro Asn Gln Arg Arg Val Tyr Ile Ser Tyr Leu
            100                 105                 110

Asp Ser Val His Phe Phe Arg Pro Lys Cys Leu Arg Thr Ala Val Tyr
        115                 120                 125

His Glu Ile Leu Ile Gly Tyr Leu Glu Tyr Val Lys Lys Leu Gly Tyr
    130                 135                 140

Thr Thr Gly His Ile Trp Ala Cys Pro Pro Ser Glu Gly Asp Asp Tyr
145                 150                 155                 160

Ile Phe His Cys His Pro Pro Asp Gln Lys Ile Pro Lys Pro Lys Arg
                165                 170                 175

Leu Gln Glu Trp Tyr Lys Lys Met Leu Asp Lys Ala Val Ser Glu Arg
            180                 185                 190

Ile Val His Asp Tyr Lys Asp Ile Phe Lys Gln Ala Thr Glu Asp Arg
        195                 200                 205
```

```
Leu Thr Ser Ala Lys Glu Leu Pro Tyr Phe Glu Gly Asp Phe Trp Pro
    210                 215                 220

Asn Val Leu Glu Glu Ser Ile Lys Glu Leu Glu Gln Glu Glu Glu Glu
225                 230                 235                 240

Arg Lys Arg Glu Glu Asn Thr Ser Asn Glu Ser Thr Asp Val Thr Lys
                245                 250                 255

Gly Asp Ser Lys Asn Ala Lys Lys Lys Asn Asn Lys Lys Thr Ser Lys
            260                 265                 270

Asn Lys Ser Ser Leu Ser Arg Gly Asn Lys Lys Lys Pro Gly Met Pro
        275                 280                 285

Asn Val Ser Asn Asp Leu Ser Gln Lys Leu Tyr Ala Thr Met Glu Lys
    290                 295                 300

His Lys Glu Val Phe Phe Val Ile Arg Leu Ile Ala Gly Pro Ala Ala
305                 310                 315                 320

Asn Ser Leu Pro Pro Ile Val Asp Pro Asp Pro Leu Ile Pro Cys Asp
                325                 330                 335

Leu Met Asp Gly Arg Asp Ala Phe Leu Thr Leu Ala Arg Asp Lys His
            340                 345                 350

Leu Glu Phe Ser Ser Leu Arg Arg Ala Gln Trp Ser Thr Met Cys Met
        355                 360                 365

Leu Val Glu Leu His Thr Gln Ser Gln
    370                 375


<210>  22
<211>  2136
```

<212> PRT
<213> Homo sapiens

<400> 22

```
Met Ser Arg Ser Arg His Ala Arg Pro Ser Arg Leu Val Arg Lys Glu
1               5                  10                  15

Asp Val Asn Lys Lys Lys Lys Asn Ser Gln Leu Arg Lys Thr Thr Lys
            20                  25                  30

Gly Ala Asn Lys Asn Val Ala Ser Val Lys Thr Leu Ser Pro Gly Lys
        35                  40                  45

Leu Lys Gln Leu Ile Gln Glu Arg Asp Val Lys Lys Lys Thr Glu Pro
    50                  55                  60

Lys Pro Pro Val Pro Val Arg Ser Leu Leu Thr Arg Ala Gly Ala Ala
65                  70                  75                  80

Arg Met Asn Leu Asp Arg Thr Glu Val Leu Phe Gln Asn Pro Glu Ser
                85                  90                  95

Leu Thr Cys Asn Gly Phe Thr Met Ala Leu Arg Ser Thr Ser Leu Ser
            100                 105                 110

Arg Arg Leu Ser Gln Pro Pro Leu Val Val Ala Lys Ser Lys Lys Val
        115                 120                 125

Pro Leu Ser Lys Gly Leu Glu Lys Gln His Asp Cys Asp Tyr Lys Ile
    130                 135                 140

Leu Pro Ala Leu Gly Val Lys His Ser Glu Asn Asp Ser Val Pro Met
145                 150                 155                 160

Gln Asp Thr Gln Val Leu Pro Asp Ile Glu Thr Leu Ile Gly Val Gln
                165                 170                 175
```

```
Asn Pro Ser Leu Leu Lys Gly Lys Ser Gln Glu Thr Thr Gln Phe Trp
            180                 185                 190

Ser Gln Arg Val Glu Asp Ser Lys Ile Asn Ile Pro Thr His Ser Gly
        195                 200                 205

Pro Ala Ala Glu Ile Leu Pro Gly Pro Leu Glu Gly Thr Arg Cys Gly
    210                 215                 220

Glu Gly Leu Phe Ser Glu Glu Thr Leu Asn Asp Thr Ser Gly Ser Pro
225                 230                 235                 240

Lys Met Phe Ala Gln Asp Thr Val Cys Ala Pro Phe Pro Gln Arg Ala
                245                 250                 255

Thr Pro Lys Val Thr Ser Gln Gly Asn Pro Ser Ile Gln Leu Glu Glu
            260                 265                 270

Leu Gly Ser Arg Val Glu Ser Leu Lys Leu Ser Asp Ser Tyr Leu Asp
        275                 280                 285

Pro Ile Lys Ser Glu His Asp Cys Tyr Pro Thr Ser Ser Leu Asn Lys
    290                 295                 300

Val Ile Pro Asp Leu Asn Leu Arg Asn Cys Leu Ala Leu Gly Gly Ser
305                 310                 315                 320

Thr Ser Pro Thr Ser Val Ile Lys Phe Leu Leu Ala Gly Ser Lys Gln
                325                 330                 335

Ala Thr Leu Gly Ala Lys Pro Asp His Gln Glu Ala Phe Glu Ala Thr
            340                 345                 350

Ala Asn Gln Gln Glu Val Ser Asp Thr Thr Ser Phe Leu Gly Gln Ala
        355                 360                 365
```

```
Phe Gly Ala Ile Pro His Gln Trp Glu Leu Pro Gly Ala Asp Pro Val
    370                 375                 380

His Gly Glu Ala Leu Gly Glu Thr Pro Asp Leu Pro Glu Ile Pro Gly
385                 390                 395                 400

Ala Ile Pro Val Gln Gly Glu Val Phe Gly Thr Ile Leu Asp Gln Gln
                405                 410                 415

Glu Thr Leu Gly Met Ser Gly Ser Val Val Pro Asp Leu Pro Val Phe
            420                 425                 430

Leu Pro Val Pro Pro Asn Pro Ile Ala Thr Phe Asn Ala Pro Ser Lys
        435                 440                 445

Trp Pro Glu Pro Gln Ser Thr Val Ser Tyr Gly Leu Ala Val Gln Gly
    450                 455                 460

Ala Ile Gln Ile Leu Pro Leu Gly Ser Gly His Thr Pro Gln Ser Ser
465                 470                 475                 480

Ser Asn Ser Glu Lys Asn Ser Leu Pro Pro Val Met Ala Ile Ser Asn
                485                 490                 495

Val Glu Asn Glu Lys Gln Val His Ile Ser Phe Leu Pro Ala Asn Thr
            500                 505                 510

Gln Gly Phe Pro Leu Ala Pro Glu Arg Gly Leu Phe His Ala Ser Leu
        515                 520                 525

Gly Ile Ala Gln Leu Ser Gln Ala Gly Pro Ser Lys Ser Asp Arg Gly
    530                 535                 540

Ser Ser Gln Val Ser Val Thr Ser Thr Val His Val Val Asn Thr Thr
545                 550                 555                 560
```

```
Val Val Thr Met Pro Val Pro Met Val Ser Thr Ser Ser Ser Ser Tyr
                565                 570                 575

Thr Thr Leu Leu Pro Thr Leu Glu Lys Lys Lys Arg Lys Arg Cys Gly
            580                 585                 590

Val Cys Glu Pro Cys Gln Gln Lys Thr Asn Cys Gly Glu Cys Thr Tyr
        595                 600                 605

Cys Lys Asn Arg Lys Asn Ser His Gln Ile Cys Lys Lys Arg Lys Cys
    610                 615                 620

Glu Glu Leu Lys Lys Lys Pro Ser Val Val Val Pro Leu Glu Val Ile
625                 630                 635                 640

Lys Glu Asn Lys Arg Pro Gln Arg Glu Lys Lys Pro Lys Val Leu Lys
                645                 650                 655

Ala Asp Phe Asp Asn Lys Pro Val Asn Gly Pro Lys Ser Glu Ser Met
            660                 665                 670

Asp Tyr Ser Arg Cys Gly His Gly Glu Glu Gln Lys Leu Glu Leu Asn
        675                 680                 685

Pro His Thr Val Glu Asn Val Thr Lys Asn Glu Asp Ser Met Thr Gly
    690                 695                 700

Ile Glu Val Glu Lys Trp Thr Gln Asn Lys Lys Ser Gln Leu Thr Asp
705                 710                 715                 720

His Val Lys Gly Asp Phe Ser Ala Asn Val Pro Glu Ala Glu Lys Ser
                725                 730                 735

Lys Asn Ser Glu Val Asp Lys Lys Arg Thr Lys Ser Pro Lys Leu Phe
            740                 745                 750
```

```
Val Gln Thr Val Arg Asn Gly Ile Lys His Val His Cys Leu Pro Ala
        755                 760                 765


Glu Thr Asn Val Ser Phe Lys Lys Phe Asn Ile Glu Glu Phe Gly Lys
    770                 775                 780


Thr Leu Glu Asn Asn Ser Tyr Lys Phe Leu Lys Asp Thr Ala Asn His
785                 790                 795                 800


Lys Asn Ala Met Ser Ser Val Ala Thr Asp Met Ser Cys Asp His Leu
                805                 810                 815


Lys Gly Arg Ser Asn Val Leu Val Phe Gln Gln Pro Gly Phe Asn Cys
            820                 825                 830


Ser Ser Ile Pro His Ser Ser His Ser Ile Ile Asn His His Ala Ser
        835                 840                 845


Ile His Asn Glu Gly Asp Gln Pro Lys Thr Pro Glu Asn Ile Pro Ser
    850                 855                 860


Lys Glu Pro Lys Asp Gly Ser Pro Val Gln Pro Ser Leu Leu Ser Leu
865                 870                 875                 880


Met Lys Asp Arg Arg Leu Thr Leu Glu Gln Val Val Ala Ile Glu Ala
                885                 890                 895


Leu Thr Gln Leu Ser Glu Ala Pro Ser Glu Asn Ser Ser Pro Ser Lys
            900                 905                 910


Ser Glu Lys Asp Glu Glu Ser Glu Gln Arg Thr Ala Ser Leu Leu Asn
        915                 920                 925


Ser Cys Lys Ala Ile Leu Tyr Thr Val Arg Lys Asp Leu Gln Asp Pro
    930                 935                 940
```

```
Asn Leu Gln Gly Glu Pro Pro Lys Leu Asn His Cys Pro Ser Leu Glu
945                 950                 955                 960


Lys Gln Ser Ser Cys Asn Thr Val Val Phe Asn Gly Gln Thr Thr Thr
                965                 970                 975


Leu Ser Asn Ser His Ile Asn Ser Ala Thr Asn Gln Ala Ser Thr Lys
            980                 985                 990


Ser His Glu Tyr Ser Lys Val Thr  Asn Ser Leu Ser Leu  Phe Ile Pro
        995                 1000                1005


Lys Ser  Asn Ser Ser Lys Ile  Asp Thr Asn Lys Ser  Ile Ala Gln
    1010                1015                1020


Gly Ile  Ile Thr Leu Asp Asn  Cys Ser Asn Asp Leu  His Gln Leu
    1025                1030                1035


Pro Pro  Arg Asn Asn Glu Val  Glu Tyr Cys Asn Gln  Leu Leu Asp
    1040                1045                1050


Ser Ser  Lys Lys Leu Asp Ser  Asp Asp Leu Ser Cys  Gln Asp Ala
    1055                1060                1065


Thr His  Thr Gln Ile Glu Glu  Asp Val Ala Thr Gln  Leu Thr Gln
    1070                1075                1080


Leu Ala  Ser Ile Ile Lys Ile  Asn Tyr Ile Lys Pro  Glu Asp Lys
    1085                1090                1095


Lys Val  Glu Ser Thr Pro Thr  Ser Leu Val Thr Cys  Asn Val Gln
    1100                1105                1110


Gln Lys  Tyr Asn Gln Glu Lys  Gly Thr Ile Gln Gln  Lys Pro Pro
    1115                1120                1125
```

```
Ser Ser  Val His Asn Asn His  Gly Ser Ser Leu Thr  Lys Gln Lys
    1130                1135                1140


Asn Pro  Thr Gln Lys Lys Thr  Lys Ser Thr Pro Ser  Arg Asp Arg
    1145                1150                1155


Arg Lys  Lys Lys Pro Thr Val  Val Ser Tyr Gln Glu  Asn Asp Arg
    1160                1165                1170


Gln Lys  Trp Glu Lys Leu Ser  Tyr Met Tyr Gly Thr  Ile Cys Asp
    1175                1180                1185


Ile Trp  Ile Ala Ser Lys Phe  Gln Asn Phe Gly Gln  Phe Cys Pro
    1190                1195                1200


His Asp  Phe Pro Thr Val Phe  Gly Lys Ile Ser Ser  Ser Thr Lys
    1205                1210                1215


Ile Trp  Lys Pro Leu Ala Gln  Thr Arg Ser Ile Met  Gln Pro Lys
    1220                1225                1230


Thr Val  Phe Pro Pro Leu Thr  Gln Ile Lys Leu Gln  Arg Tyr Pro
    1235                1240                1245


Glu Ser  Ala Glu Glu Lys Val  Lys Val Glu Pro Leu  Asp Ser Leu
    1250                1255                1260


Ser Leu  Phe His Leu Lys Thr  Glu Ser Asn Gly Lys  Ala Phe Thr
    1265                1270                1275


Asp Lys  Ala Tyr Asn Ser Gln  Val Gln Leu Thr Val  Asn Ala Asn
    1280                1285                1290


Gln Lys  Ala His Pro Leu Thr  Gln Pro Ser Ser Pro  Pro Asn Gln
    1295                1300                1305
```

```
Cys Ala  Asn Val Met Ala Gly  Asp Asp Gln Ile Arg  Phe Gln Gln
    1310                1315                1320


Val Val  Lys Glu Gln Leu Met  His Gln Arg Leu Pro  Thr Leu Pro
    1325                1330                1335


Gly Ile  Ser His Glu Thr Pro  Leu Pro Glu Ser Ala  Leu Thr Leu
    1340                1345                1350


Arg Asn  Val Asn Val Val Cys  Ser Gly Gly Ile Thr  Val Val Ser
    1355                1360                1365


Thr Lys  Ser Glu Glu Glu Val  Cys Ser Ser Ser Phe  Gly Thr Ser
    1370                1375                1380


Glu Phe  Ser Thr Val Asp Ser  Ala Gln Lys Asn Phe  Asn Asp Tyr
    1385                1390                1395


Ala Met  Asn Phe Phe Thr Asn  Pro Thr Lys Asn Leu  Val Ser Ile
    1400                1405                1410


Thr Lys  Asp Ser Glu Leu Pro  Thr Cys Ser Cys Leu  Asp Arg Val
    1415                1420                1425


Ile Gln  Lys Asp Lys Gly Pro  Tyr Tyr Thr His Leu  Gly Ala Gly
    1430                1435                1440


Pro Ser  Val Ala Ala Val Arg  Glu Ile Met Glu Asn  Arg Tyr Gly
    1445                1450                1455


Gln Lys  Gly Asn Ala Ile Arg  Ile Glu Ile Val Val  Tyr Thr Gly
    1460                1465                1470


Lys Glu  Gly Lys Ser Ser His  Gly Cys Pro Ile Ala  Lys Trp Val
    1475                1480                1485
```

```
Leu Arg  Arg Ser Ser Asp Glu  Glu Lys Val Leu Cys  Leu Val Arg
    1490                1495                1500

Gln Arg  Thr Gly His His Cys  Pro Thr Ala Val Met  Val Val Leu
    1505                1510                1515

Ile Met  Val Trp Asp Gly Ile  Pro Leu Pro Met Ala  Asp Arg Leu
    1520                1525                1530

Tyr Thr  Glu Leu Thr Glu Asn  Leu Lys Ser Tyr Asn  Gly His Pro
    1535                1540                1545

Thr Asp  Arg Arg Cys Thr Leu  Asn Glu Asn Arg Thr  Cys Thr Cys
    1550                1555                1560

Gln Gly  Ile Asp Pro Glu Thr  Cys Gly Ala Ser Phe  Ser Phe Gly
    1565                1570                1575

Cys Ser  Trp Ser Met Tyr Phe  Asn Gly Cys Lys Phe  Gly Arg Ser
    1580                1585                1590

Pro Ser  Pro Arg Arg Phe Arg  Ile Asp Pro Ser Ser  Pro Leu His
    1595                1600                1605

Glu Lys  Asn Leu Glu Asp Asn  Leu Gln Ser Leu Ala  Thr Arg Leu
    1610                1615                1620

Ala Pro  Ile Tyr Lys Gln Tyr  Ala Pro Val Ala Tyr  Gln Asn Gln
    1625                1630                1635

Val Glu  Tyr Glu Asn Val Ala  Arg Glu Cys Arg Leu  Gly Ser Lys
    1640                1645                1650

Glu Gly  Arg Pro Phe Ser Gly  Val Thr Ala Cys Leu  Asp Phe Cys
    1655                1660                1665
```

```
Ala His  Pro His Arg Asp Ile  His Asn Met Asn Asn  Gly Ser Thr
    1670                1675                1680

Val Val  Cys Thr Leu Thr Arg  Glu Asp Asn Arg Ser  Leu Gly Val
    1685                1690                1695

Ile Pro  Gln Asp Glu Gln Leu  His Val Leu Pro Leu  Tyr Lys Leu
    1700                1705                1710

Ser Asp  Thr Asp Glu Phe Gly  Ser Lys Glu Gly Met  Glu Ala Lys
    1715                1720                1725

Ile Lys  Ser Gly Ala Ile Glu  Val Leu Ala Pro Arg  Arg Lys Lys
    1730                1735                1740

Arg Thr  Cys Phe Thr Gln Pro  Val Pro Arg Ser Gly  Lys Lys Arg
    1745                1750                1755

Ala Ala  Met Met Thr Glu Val  Leu Ala His Lys Ile  Arg Ala Val
    1760                1765                1770

Glu Lys  Lys Pro Ile Pro Arg  Ile Lys Arg Lys Asn  Asn Ser Thr
    1775                1780                1785

Thr Thr  Asn Asn Ser Lys Pro  Ser Ser Leu Pro Thr  Leu Gly Ser
    1790                1795                1800

Asn Thr  Glu Thr Val Gln Pro  Glu Val Lys Ser Glu  Thr Glu Pro
    1805                1810                1815

His Phe  Ile Leu Lys Ser Ser  Asp Asn Thr Lys Thr  Tyr Ser Leu
    1820                1825                1830

Met Pro  Ser Ala Pro His Pro  Val Lys Glu Ala Ser  Pro Gly Phe
    1835                1840                1845
```

```
Ser Trp  Ser Pro Lys Thr Ala  Ser Ala Thr Pro Ala  Pro Leu Lys
    1850                 1855                 1860


Asn Asp  Ala Thr Ala Ser Cys  Gly Phe Ser Glu Arg  Ser Ser Thr
    1865                 1870                 1875


Pro His  Cys Thr Met Pro Ser  Gly Arg Leu Ser Gly  Ala Asn Ala
    1880                 1885                 1890


Ala Ala  Ala Asp Gly Pro Gly  Ile Ser Gln Leu Gly  Glu Val Ala
    1895                 1900                 1905


Pro Leu  Pro Thr Leu Ser Ala  Pro Val Met Glu Pro  Leu Ile Asn
    1910                 1915                 1920


Ser Glu  Pro Ser Thr Gly Val  Thr Glu Pro Leu Thr  Pro His Gln
    1925                 1930                 1935


Pro Asn  His Gln Pro Ser Phe  Leu Thr Ser Pro Gln  Asp Leu Ala
    1940                 1945                 1950


Ser Ser  Pro Met Glu Glu Asp  Glu Gln His Ser Glu  Ala Asp Glu
    1955                 1960                 1965


Pro Pro  Ser Asp Glu Pro Leu  Ser Asp Asp Pro Leu  Ser Pro Ala
    1970                 1975                 1980


Glu Glu  Lys Leu Pro His Ile  Asp Glu Tyr Trp Ser  Asp Ser Glu
    1985                 1990                 1995


His Ile  Phe Leu Asp Ala Asn  Ile Gly Gly Val Ala  Ile Ala Pro
    2000                 2005                 2010


Ala His  Gly Ser Val Leu Ile  Glu Cys Ala Arg Arg  Glu Leu His
    2015                 2020                 2025
```

```
Ala Thr  Thr Pro Val Glu His  Pro Asn Arg Asn His  Pro Thr Arg
    2030                2035                2040


Leu Ser  Leu Val Phe Tyr Gln  His Lys Asn Leu Asn  Lys Pro Gln
    2045                2050                2055


His Gly  Phe Glu Leu Asn Lys  Ile Lys Phe Glu Ala  Lys Glu Ala
    2060                2065                2070


Lys Asn  Lys Lys Met Lys Ala  Ser Glu Gln Lys Asp  Gln Ala Ala
    2075                2080                2085


Asn Glu  Gly Pro Glu Gln Ser  Ser Glu Val Asn Glu  Leu Asn Gln
    2090                2095                2100


Ile Pro  Ser His Lys Ala Leu  Thr Leu Thr His Asp  Asn Val Val
    2105                2110                2115


Thr Val  Ser Pro Tyr Ala Leu  Thr His Val Ala Gly  Pro Tyr Asn
    2120                2125                2130


His Trp  Val
    2135


<210>  23
<211>  552
<212>  PRT
<213>  Mus musculus

<400>  23

Gly Met Asp Val Thr Leu Leu Glu Ala Arg Asp Arg Val Gly Gly Arg
1               5                  10                  15


Val Ala Thr Phe Arg Lys Gly Asn Tyr Val Ala Asp Leu Gly Ala Met
            20                  25                  30
```

```
Val Val Thr Gly Leu Gly Gly Asn Pro Met Ala Val Val Ser Lys Gln
        35                  40                  45

Val Asn Met Glu Leu Ala Lys Ile Lys Gln Lys Cys Pro Leu Tyr Glu
    50                  55                  60

Ala Asn Gly Gln Ala Val Pro Lys Glu Lys Asp Glu Met Val Glu Gln
65                  70                  75                  80

Glu Phe Asn Arg Leu Leu Glu Ala Thr Ser Tyr Leu Ser His Gln Leu
                85                  90                  95

Asp Phe Asn Val Leu Asn Asn Lys Pro Val Ser Leu Gly Gln Ala Leu
            100                 105                 110

Glu Val Val Ile Gln Leu Gln Glu Lys His Val Lys Asp Glu Gln Ile
        115                 120                 125

Glu His Trp Lys Lys Ile Val Lys Thr Gln Glu Glu Leu Lys Glu Leu
    130                 135                 140

Leu Asn Lys Met Val Asn Leu Lys Glu Lys Ile Lys Glu Leu His Gln
145                 150                 155                 160

Gln Tyr Lys Glu Ala Ser Glu Val Lys Pro Pro Arg Asp Ile Thr Ala
                165                 170                 175

Glu Phe Leu Val Lys Ser Lys His Arg Asp Leu Thr Ala Leu Cys Lys
            180                 185                 190

Glu Tyr Asp Glu Leu Ala Glu Thr Gln Gly Lys Leu Glu Glu Lys Leu
        195                 200                 205

Gln Glu Leu Glu Ala Asn Pro Pro Ser Asp Val Tyr Leu Ser Ser Arg
    210                 215                 220
```

```
Asp Arg Gln Ile Leu Asp Trp His Phe Ala Asn Leu Glu Phe Ala Asn
225                 230                 235                 240


Ala Thr Pro Leu Ser Thr Leu Ser Leu Lys His Trp Asp Gln Asp Asp
                245                 250                 255


Asp Phe Glu Phe Thr Gly Ser His Leu Thr Val Arg Asn Gly Tyr Ser
            260                 265                 270


Cys Val Pro Val Ala Leu Ala Glu Gly Leu Asp Ile Lys Leu Asn Thr
        275                 280                 285


Ala Val Arg Gln Val Arg Tyr Thr Ala Ser Gly Cys Glu Val Ile Ala
    290                 295                 300


Val Asn Thr Arg Ser Thr Ser Gln Thr Phe Ile Tyr Lys Cys Asp Ala
305                 310                 315                 320


Val Leu Cys Thr Leu Pro Leu Gly Val Leu Lys Gln Gln Pro Pro Ala
                325                 330                 335


Val Gln Phe Val Pro Pro Leu Pro Glu Trp Lys Thr Ser Ala Val Gln
            340                 345                 350


Arg Met Gly Phe Gly Asn Leu Asn Lys Val Val Leu Cys Phe Asp Arg
        355                 360                 365


Val Phe Trp Asp Pro Ser Val Asn Leu Phe Gly His Val Gly Ser Thr
    370                 375                 380


Thr Ala Ser Arg Gly Glu Leu Phe Leu Phe Trp Asn Leu Tyr Lys Ala
385                 390                 395                 400


Pro Ile Leu Leu Ala Leu Val Ala Gly Glu Ala Ala Gly Ile Met Glu
                405                 410                 415
```

```
Asn Ile Ser Asp Asp Val Ile Val Gly Arg Cys Leu Ala Ile Leu Lys
            420                 425                 430


Gly Ile Phe Gly Ser Ser Ala Val Pro Gln Pro Lys Glu Thr Val Val
        435                 440                 445


Ser Arg Trp Arg Ala Asp Pro Trp Ala Arg Gly Ser Tyr Ser Tyr Val
    450                 455                 460


Ala Ala Gly Ser Ser Gly Asn Asp Tyr Asp Leu Met Ala Gln Pro Ile
465                 470                 475                 480


Thr Pro Gly Pro Ser Ile Pro Gly Ala Pro Gln Pro Ile Pro Arg Leu
                485                 490                 495


Phe Phe Ala Gly Glu His Thr Ile Arg Asn Tyr Pro Ala Thr Val His
            500                 505                 510


Gly Ala Leu Leu Ser Gly Leu Arg Glu Ala Gly Arg Ile Ala Asp Gln
        515                 520                 525


Phe Leu Gly Ala Met Tyr Thr Leu Pro Arg Gln Ala Thr Pro Gly Val
    530                 535                 540


Pro Ala Gln Gln Ser Pro Ser Met
545                 550


<210>  24
<211>  191
<212>  PRT
<213>  Homo sapiens

<400>  24

Met Gly Gly Ser Gly Ser Arg Leu Ser Lys Glu Leu Leu Ala Glu Tyr
1               5                   10                  15


Gln Asp Leu Thr Phe Leu Thr Lys Gln Glu Ile Leu Leu Ala His Arg
```

```
            20                  25                  30

Arg Phe Cys Glu Leu Leu Pro Gln Glu Gln Arg Ser Val Glu Ser Ser
        35                  40                  45

Leu Arg Ala Gln Val Pro Phe Glu Gln Ile Leu Ser Leu Pro Glu Leu
    50                  55                  60

Lys Ala Asn Pro Phe Lys Glu Arg Ile Cys Arg Val Phe Ser Thr Ser
65                  70                  75                  80

Pro Ala Lys Asp Ser Leu Ser Phe Glu Asp Phe Leu Asp Leu Leu Ser
                85                  90                  95

Val Phe Ser Asp Thr Ala Thr Pro Asp Ile Lys Ser His Tyr Ala Phe
            100                 105                 110

Arg Ile Phe Asp Phe Asp Asp Asp Gly Thr Leu Asn Arg Glu Asp Leu
        115                 120                 125

Ser Arg Leu Val Asn Cys Leu Thr Gly Glu Gly Glu Asp Thr Arg Leu
    130                 135                 140

Ser Ala Ser Glu Met Lys Gln Leu Ile Asp Asn Ile Leu Glu Glu Ser
145                 150                 155                 160

Asp Ile Asp Arg Asp Gly Thr Ile Asn Leu Ser Glu Phe Gln His Val
                165                 170                 175

Ile Ser Arg Ser Pro Asp Phe Ala Ser Ser Phe Lys Ile Val Leu
            180                 185                 190


<210>  25
<211>  654
<212>  PRT
<213>  Homo sapiens
```

<400> 25

```
Met Asn Gln Pro Gln Arg Met Ala Pro Val Gly Thr Asp Lys Glu Leu
1               5                  10                  15

Ser Asp Leu Leu Asp Phe Ser Met Met Phe Pro Leu Pro Val Thr Asn
            20                  25                  30

Gly Lys Gly Arg Pro Ala Ser Leu Ala Gly Ala Gln Phe Gly Gly Ser
        35                  40                  45

Gly Leu Glu Asp Arg Pro Ser Ser Gly Ser Trp Gly Ser Gly Asp Gln
    50                  55                  60

Ser Ser Ser Ser Phe Asp Pro Ser Arg Thr Phe Ser Glu Gly Thr His
65                  70                  75                  80

Phe Thr Glu Ser His Ser Ser Leu Ser Ser Ser Thr Phe Leu Gly Pro
                85                  90                  95

Gly Leu Gly Gly Lys Ser Gly Glu Arg Gly Ala Tyr Ala Ser Phe Gly
            100                 105                 110

Arg Asp Ala Gly Val Gly Gly Leu Thr Gln Ala Gly Phe Leu Ser Gly
        115                 120                 125

Glu Leu Ala Leu Asn Ser Pro Gly Pro Leu Ser Pro Ser Gly Met Lys
    130                 135                 140

Gly Thr Ser Gln Tyr Tyr Pro Ser Tyr Ser Gly Ser Ser Arg Arg Arg
145                 150                 155                 160

Ala Ala Asp Gly Ser Leu Asp Thr Gln Pro Lys Lys Val Arg Lys Val
                165                 170                 175

Pro Pro Gly Leu Pro Ser Ser Val Tyr Pro Pro Ser Ser Gly Glu Asp
            180                 185                 190
```

```
Tyr Gly Arg Asp Ala Thr Ala Tyr Pro Ser Ala Lys Thr Pro Ser Ser
        195                 200                 205

Thr Tyr Pro Ala Pro Phe Tyr Val Ala Asp Gly Ser Leu His Pro Ser
    210                 215                 220

Ala Glu Leu Trp Ser Pro Pro Gly Gln Ala Gly Phe Gly Pro Met Leu
225                 230                 235                 240

Gly Gly Gly Ser Ser Pro Leu Pro Leu Pro Pro Gly Ser Gly Pro Val
                245                 250                 255

Gly Ser Ser Gly Ser Ser Ser Thr Phe Gly Gly Leu His Gln His Glu
            260                 265                 270

Arg Met Gly Tyr Gln Leu His Gly Ala Glu Val Asn Gly Gly Leu Pro
        275                 280                 285

Ser Ala Ser Ser Phe Ser Ser Ala Pro Gly Ala Thr Tyr Gly Gly Val
    290                 295                 300

Ser Ser His Thr Pro Pro Val Ser Gly Ala Asp Ser Leu Leu Gly Ser
305                 310                 315                 320

Arg Gly Thr Thr Ala Gly Ser Ser Gly Asp Ala Leu Gly Lys Ala Leu
                325                 330                 335

Ala Ser Ile Tyr Ser Pro Asp His Ser Ser Asn Asn Phe Ser Ser Ser
            340                 345                 350

Pro Ser Thr Pro Val Gly Ser Pro Gln Gly Leu Ala Gly Thr Ser Gln
        355                 360                 365

Trp Pro Arg Ala Gly Ala Pro Gly Ala Leu Ser Pro Ser Tyr Asp Gly
    370                 375                 380
```

```
Gly Leu His Gly Leu Gln Ser Lys Ile Glu Asp His Leu Asp Glu Ala
385                 390                 395                 400

Ile His Val Leu Arg Ser His Ala Val Gly Thr Ala Gly Asp Met His
                405                 410                 415

Thr Leu Leu Pro Gly His Gly Ala Leu Ala Ser Gly Phe Thr Gly Pro
            420                 425                 430

Met Ser Leu Gly Gly Arg His Ala Gly Leu Val Gly Gly Ser His Pro
        435                 440                 445

Glu Asp Gly Leu Ala Gly Ser Thr Ser Leu Met His Asn His Ala Ala
    450                 455                 460

Leu Pro Ser Gln Pro Gly Thr Leu Pro Asp Leu Ser Arg Pro Pro Asp
465                 470                 475                 480

Ser Tyr Ser Gly Leu Gly Arg Ala Gly Ala Thr Ala Ala Ala Ser Glu
                485                 490                 495

Ile Lys Arg Glu Glu Lys Glu Asp Glu Glu Asn Thr Ser Ala Ala Asp
            500                 505                 510

His Ser Glu Glu Glu Lys Lys Glu Leu Lys Ala Pro Arg Ala Arg Thr
        515                 520                 525

Ser Pro Asp Glu Asp Glu Asp Asp Leu Leu Pro Pro Glu Gln Lys Ala
    530                 535                 540

Glu Arg Glu Lys Glu Arg Arg Val Ala Asn Asn Ala Arg Glu Arg Leu
545                 550                 555                 560

Arg Val Arg Asp Ile Asn Glu Ala Phe Lys Glu Leu Gly Arg Met Cys
                565                 570                 575
```

```
Gln Leu His Leu Asn Ser Glu Lys Pro Gln Thr Lys Leu Leu Ile Leu
            580                 585                 590

His Gln Ala Val Ser Val Ile Leu Asn Leu Glu Gln Gln Val Arg Glu
        595                 600                 605

Arg Asn Leu Asn Pro Lys Ala Ala Cys Leu Lys Arg Arg Glu Glu Glu
    610                 615                 620

Lys Val Ser Gly Val Val Gly Asp Pro Gln Met Val Leu Ser Ala Pro
625                 630                 635                 640

His Pro Gly Leu Ser Glu Ala His Asn Pro Ala Gly His Met
                645                 650

<210>  26
<211>  1663
<212>  PRT
<213>  Homo sapiens

<400>  26

Met Gly Pro Thr Ser Gly Pro Ser Leu Leu Leu Leu Leu Leu Thr His
1               5                   10                  15

Leu Pro Leu Ala Leu Gly Ser Pro Met Tyr Ser Ile Ile Thr Pro Asn
            20                  25                  30

Ile Leu Arg Leu Glu Ser Glu Glu Thr Met Val Leu Glu Ala His Asp
        35                  40                  45

Ala Gln Gly Asp Val Pro Val Thr Val Thr Val His Asp Phe Pro Gly
    50                  55                  60

Lys Lys Leu Val Leu Ser Ser Glu Lys Thr Val Leu Thr Pro Ala Thr
65                  70                  75                  80
```

```
Asn His Met Gly Asn Val Thr Phe Thr Ile Pro Ala Asn Arg Glu Phe
                85                  90                  95


Lys Ser Glu Lys Gly Arg Asn Lys Phe Val Thr Val Gln Ala Thr Phe
            100                 105                 110


Gly Thr Gln Val Val Glu Lys Val Val Leu Val Ser Leu Gln Ser Gly
        115                 120                 125


Tyr Leu Phe Ile Gln Thr Asp Lys Thr Ile Tyr Thr Pro Gly Ser Thr
    130                 135                 140


Val Leu Tyr Arg Ile Phe Thr Val Asn His Lys Leu Leu Pro Val Gly
145                 150                 155                 160


Arg Thr Val Met Val Asn Ile Glu Asn Pro Glu Gly Ile Pro Val Lys
                165                 170                 175


Gln Asp Ser Leu Ser Ser Gln Asn Gln Leu Gly Val Leu Pro Leu Ser
            180                 185                 190


Trp Asp Ile Pro Glu Leu Val Asn Met Gly Gln Trp Lys Ile Arg Ala
        195                 200                 205


Tyr Tyr Glu Asn Ser Pro Gln Gln Val Phe Ser Thr Glu Phe Glu Val
    210                 215                 220


Lys Glu Tyr Val Leu Pro Ser Phe Glu Val Ile Val Glu Pro Thr Glu
225                 230                 235                 240


Lys Phe Tyr Tyr Ile Tyr Asn Glu Lys Gly Leu Glu Val Thr Ile Thr
                245                 250                 255


Ala Arg Phe Leu Tyr Gly Lys Lys Val Glu Gly Thr Ala Phe Val Ile
            260                 265                 270
```

```
Phe Gly Ile Gln Asp Gly Glu Gln Arg Ile Ser Leu Pro Glu Ser Leu
        275                 280                 285


Lys Arg Ile Pro Ile Glu Asp Gly Ser Gly Glu Val Val Leu Ser Arg
    290                 295                 300


Lys Val Leu Leu Asp Gly Val Gln Asn Pro Arg Ala Glu Asp Leu Val
305                 310                 315                 320


Gly Lys Ser Leu Tyr Val Ser Ala Thr Val Ile Leu His Ser Gly Ser
                325                 330                 335


Asp Met Val Gln Ala Glu Arg Ser Gly Ile Pro Ile Val Thr Ser Pro
            340                 345                 350


Tyr Gln Ile His Phe Thr Lys Thr Pro Lys Tyr Phe Lys Pro Gly Met
        355                 360                 365


Pro Phe Asp Leu Met Val Phe Val Thr Asn Pro Asp Gly Ser Pro Ala
    370                 375                 380


Tyr Arg Val Pro Val Ala Val Gln Gly Glu Asp Thr Val Gln Ser Leu
385                 390                 395                 400


Thr Gln Gly Asp Gly Val Ala Lys Leu Ser Ile Asn Thr His Pro Ser
                405                 410                 415


Gln Lys Pro Leu Ser Ile Thr Val Arg Thr Lys Lys Gln Glu Leu Ser
            420                 425                 430


Glu Ala Glu Gln Ala Thr Arg Thr Met Gln Ala Leu Pro Tyr Ser Thr
        435                 440                 445


Val Gly Asn Ser Asn Asn Tyr Leu His Leu Ser Val Leu Arg Thr Glu
    450                 455                 460
```

```
Leu Arg Pro Gly Glu Thr Leu Asn Val Asn Phe Leu Leu Arg Met Asp
465                 470                 475                 480


Arg Ala His Glu Ala Lys Ile Arg Tyr Tyr Thr Tyr Leu Ile Met Asn
                485                 490                 495


Lys Gly Arg Leu Leu Lys Ala Gly Arg Gln Val Arg Glu Pro Gly Gln
            500                 505                 510


Asp Leu Val Val Leu Pro Leu Ser Ile Thr Thr Asp Phe Ile Pro Ser
        515                 520                 525


Phe Arg Leu Val Ala Tyr Tyr Thr Leu Ile Gly Ala Ser Gly Gln Arg
    530                 535                 540


Glu Val Val Ala Asp Ser Val Trp Val Asp Val Lys Asp Ser Cys Val
545                 550                 555                 560


Gly Ser Leu Val Val Lys Ser Gly Gln Ser Glu Asp Arg Gln Pro Val
                565                 570                 575


Pro Gly Gln Gln Met Thr Leu Lys Ile Glu Gly Asp His Gly Ala Arg
            580                 585                 590


Val Val Leu Val Ala Val Asp Lys Gly Val Phe Val Leu Asn Lys Lys
        595                 600                 605


Asn Lys Leu Thr Gln Ser Lys Ile Trp Asp Val Val Glu Lys Ala Asp
    610                 615                 620


Ile Gly Cys Thr Pro Gly Ser Gly Lys Asp Tyr Ala Gly Val Phe Ser
625                 630                 635                 640


Asp Ala Gly Leu Thr Phe Thr Ser Ser Ser Gly Gln Gln Thr Ala Gln
                645                 650                 655
```

```
Arg Ala Glu Leu Gln Cys Pro Gln Pro Ala Ala Arg Arg Arg Arg Ser
            660                 665                 670

Val Gln Leu Thr Glu Lys Arg Met Asp Lys Val Gly Lys Tyr Pro Lys
        675                 680                 685

Glu Leu Arg Lys Cys Cys Glu Asp Gly Met Arg Glu Asn Pro Met Arg
    690                 695                 700

Phe Ser Cys Gln Arg Arg Thr Arg Phe Ile Ser Leu Gly Glu Ala Cys
705                 710                 715                 720

Lys Lys Val Phe Leu Asp Cys Cys Asn Tyr Ile Thr Glu Leu Arg Arg
                725                 730                 735

Gln His Ala Arg Ala Ser His Leu Gly Leu Ala Arg Ser Asn Leu Asp
            740                 745                 750

Glu Asp Ile Ile Ala Glu Glu Asn Ile Val Ser Arg Ser Glu Phe Pro
        755                 760                 765

Glu Ser Trp Leu Trp Asn Val Glu Asp Leu Lys Glu Pro Pro Lys Asn
    770                 775                 780

Gly Ile Ser Thr Lys Leu Met Asn Ile Phe Leu Lys Asp Ser Ile Thr
785                 790                 795                 800

Thr Trp Glu Ile Leu Ala Val Ser Met Ser Asp Lys Lys Gly Ile Cys
                805                 810                 815

Val Ala Asp Pro Phe Glu Val Thr Val Met Gln Asp Phe Phe Ile Asp
            820                 825                 830

Leu Arg Leu Pro Tyr Ser Val Val Arg Asn Glu Gln Val Glu Ile Arg
        835                 840                 845
```

```
Ala Val Leu Tyr Asn Tyr Arg Gln Asn Gln Glu Leu Lys Val Arg Val
    850                 855                 860

Glu Leu Leu His Asn Pro Ala Phe Cys Ser Leu Ala Thr Thr Lys Arg
865                 870                 875                 880

Arg His Gln Gln Thr Val Thr Ile Pro Pro Lys Ser Ser Leu Ser Val
                885                 890                 895

Pro Tyr Val Ile Val Pro Leu Lys Thr Gly Leu Gln Glu Val Glu Val
            900                 905                 910

Lys Ala Ala Val Tyr His His Phe Ile Ser Asp Gly Val Arg Lys Ser
        915                 920                 925

Leu Lys Val Val Pro Glu Gly Ile Arg Met Asn Lys Thr Val Ala Val
    930                 935                 940

Arg Thr Leu Asp Pro Glu Arg Leu Gly Arg Glu Gly Val Gln Lys Glu
945                 950                 955                 960

Asp Ile Pro Pro Ala Asp Leu Ser Asp Gln Val Pro Asp Thr Glu Ser
                965                 970                 975

Glu Thr Arg Ile Leu Leu Gln Gly Thr Pro Val Ala Gln Met Thr Glu
            980                 985                 990

Asp Ala Val Asp Ala Glu Arg Leu  Lys His Leu Ile Val  Thr Pro Ser
        995                 1000                1005

Gly Cys  Gly Glu Gln Asn Met  Ile Gly Met Thr Pro  Thr Val Ile
    1010                1015                1020

Ala Val  His Tyr Leu Asp Glu  Thr Glu Gln Trp Glu  Lys Phe Gly
    1025                1030                1035
```

```
Leu Glu  Lys Arg Gln Gly Ala  Leu Glu Leu Ile Lys  Lys Gly Tyr
    1040                1045                1050


Thr Gln  Gln Leu Ala Phe Arg  Gln Pro Ser Ser Ala  Phe Ala Ala
    1055                1060                1065


Phe Val  Lys Arg Ala Pro Ser  Thr Trp Leu Thr Ala  Tyr Val Val
    1070                1075                1080


Lys Val  Phe Ser Leu Ala Val  Asn Leu Ile Ala Ile  Asp Ser Gln
    1085                1090                1095


Val Leu  Cys Gly Ala Val Lys  Trp Leu Ile Leu Glu  Lys Gln Lys
    1100                1105                1110


Pro Asp  Gly Val Phe Gln Glu  Asp Ala Pro Val Ile  His Gln Glu
    1115                1120                1125


Met Ile  Gly Gly Leu Arg Asn  Asn Asn Glu Lys Asp  Met Ala Leu
    1130                1135                1140


Thr Ala  Phe Val Leu Ile Ser  Leu Gln Glu Ala Lys  Asp Ile Cys
    1145                1150                1155


Glu Glu  Gln Val Asn Ser Leu  Pro Gly Ser Ile Thr  Lys Ala Gly
    1160                1165                1170


Asp Phe  Leu Glu Ala Asn Tyr  Met Asn Leu Gln Arg  Ser Tyr Thr
    1175                1180                1185


Val Ala  Ile Ala Gly Tyr Ala  Leu Ala Gln Met Gly  Arg Leu Lys
    1190                1195                1200


Gly Pro  Leu Leu Asn Lys Phe  Leu Thr Thr Ala Lys  Asp Lys Asn
    1205                1210                1215
```

```
Arg Trp  Glu Asp Pro Gly Lys  Gln Leu Tyr Asn Val  Glu Ala Thr
    1220                 1225                 1230


Ser Tyr  Ala Leu Leu Ala Leu  Leu Gln Leu Lys Asp  Phe Asp Phe
    1235                 1240                 1245


Val Pro  Pro Val Val Arg Trp  Leu Asn Glu Gln Arg  Tyr Tyr Gly
    1250                 1255                 1260


Gly Gly  Tyr Gly Ser Thr Gln  Ala Thr Phe Met Val  Phe Gln Ala
    1265                 1270                 1275


Leu Ala  Gln Tyr Gln Lys Asp  Ala Pro Asp His Gln  Glu Leu Asn
    1280                 1285                 1290


Leu Asp  Val Ser Leu Gln Leu  Pro Ser Arg Ser Ser  Lys Ile Thr
    1295                 1300                 1305


His Arg  Ile His Trp Glu Ser  Ala Ser Leu Leu Arg  Ser Glu Glu
    1310                 1315                 1320


Thr Lys  Glu Asn Glu Gly Phe  Thr Val Thr Ala Glu  Gly Lys Gly
    1325                 1330                 1335


Gln Gly  Thr Leu Ser Val Val  Thr Met Tyr His Ala  Lys Ala Lys
    1340                 1345                 1350


Asp Gln  Leu Thr Cys Asn Lys  Phe Asp Leu Lys Val  Thr Ile Lys
    1355                 1360                 1365


Pro Ala  Pro Glu Thr Glu Lys  Arg Pro Gln Asp Ala  Lys Asn Thr
    1370                 1375                 1380


Met Ile  Leu Glu Ile Cys Thr  Arg Tyr Arg Gly Asp  Gln Asp Ala
    1385                 1390                 1395
```

```
Thr Met  Ser Ile Leu Asp Ile  Ser Met Met Thr Gly  Phe Ala Pro
    1400                1405                1410


Asp Thr  Asp Asp Leu Lys Gln  Leu Ala Asn Gly Val  Asp Arg Tyr
    1415                1420                1425


Ile Ser  Lys Tyr Glu Leu Asp  Lys Ala Phe Ser Asp  Arg Asn Thr
    1430                1435                1440


Leu Ile  Ile Tyr Leu Asp Lys  Val Ser His Ser Glu  Asp Asp Cys
    1445                1450                1455


Leu Ala  Phe Lys Val His Gln  Tyr Phe Asn Val Glu  Leu Ile Gln
    1460                1465                1470


Pro Gly  Ala Val Lys Val Tyr  Ala Tyr Tyr Asn Leu  Glu Glu Ser
    1475                1480                1485


Cys Thr  Arg Phe Tyr His Pro  Glu Lys Glu Asp Gly  Lys Leu Asn
    1490                1495                1500


Lys Leu  Cys Arg Asp Glu Leu  Cys Arg Cys Ala Glu  Glu Asn Cys
    1505                1510                1515


Phe Ile  Gln Lys Ser Asp Asp  Lys Val Thr Leu Glu  Glu Arg Leu
    1520                1525                1530


Asp Lys  Ala Cys Glu Pro Gly  Val Asp Tyr Val Tyr  Lys Thr Arg
    1535                1540                1545


Leu Val  Lys Val Gln Leu Ser  Asn Asp Phe Asp Glu  Tyr Ile Met
    1550                1555                1560


Ala Ile  Glu Gln Thr Ile Lys  Ser Gly Ser Asp Glu  Val Gln Val
    1565                1570                1575
```

```
Gly Gln  Gln Arg Thr Phe Ile  Ser Pro Ile Lys Cys  Arg Glu Ala
    1580                1585                1590


Leu Lys  Leu Glu Glu Lys Lys  His Tyr Leu Met Trp  Gly Leu Ser
    1595                1600                1605


Ser Asp  Phe Trp Gly Glu Lys  Pro Asn Leu Ser Tyr  Ile Ile Gly
    1610                1615                1620


Lys Asp  Thr Trp Val Glu His  Trp Pro Glu Glu Asp  Glu Cys Gln
    1625                1630                1635


Asp Glu  Glu Asn Gln Lys Gln  Cys Gln Asp Leu Gly  Ala Phe Thr
    1640                1645                1650


Glu Ser  Met Val Val Phe Gly  Cys Pro Asn
    1655                1660
```

<210> 27
<211> 442
<212> PRT
<213> Homo sapiens

<400> 27

```
Met Tyr Gln Ser Leu Ala Met Ala Ala Asn His Gly Pro Pro Pro Gly
1               5                   10                  15


Ala Tyr Glu Ala Gly Gly Pro Gly Ala Phe Met His Gly Ala Gly Ala
            20                  25                  30


Ala Ser Ser Pro Val Tyr Val Pro Thr Pro Arg Val Pro Ser Ser Val
        35                  40                  45


Leu Gly Leu Ser Tyr Leu Gln Gly Gly Gly Ala Gly Ser Ala Ser Gly
    50                  55                  60
```

```
Gly Ala Ser Gly Gly Ser Ser Gly Gly Ala Ala Ser Gly Ala Gly Pro
65                  70                  75                  80


Gly Thr Gln Gln Gly Ser Pro Gly Trp Ser Gln Ala Gly Ala Asp Gly
                85                  90                  95


Ala Ala Tyr Thr Pro Pro Pro Val Ser Pro Arg Phe Ser Phe Pro Gly
            100                 105                 110


Thr Thr Gly Ser Leu Ala Ala Ala Ala Ala Ala Ala Ala Ala Arg Glu
        115                 120                 125


Ala Ala Ala Tyr Ser Ser Gly Gly Gly Ala Ala Gly Ala Gly Leu Ala
    130                 135                 140


Gly Arg Glu Gln Tyr Gly Arg Ala Gly Phe Ala Gly Ser Tyr Ser Ser
145                 150                 155                 160


Pro Tyr Pro Ala Tyr Met Ala Asp Val Gly Ala Ser Trp Ala Ala Ala
                165                 170                 175


Ala Ala Ala Ser Ala Gly Pro Phe Asp Ser Pro Val Leu His Ser Leu
            180                 185                 190


Pro Gly Arg Ala Asn Pro Ala Ala Arg His Pro Asn Leu Asp Met Phe
        195                 200                 205


Asp Asp Phe Ser Glu Gly Arg Glu Cys Val Asn Cys Gly Ala Met Ser
    210                 215                 220


Thr Pro Leu Trp Arg Arg Asp Gly Thr Gly His Tyr Leu Cys Asn Ala
225                 230                 235                 240


Cys Gly Leu Tyr His Lys Met Asn Gly Ile Asn Arg Pro Leu Ile Lys
                245                 250                 255
```

```
Pro Gln Arg Arg Leu Ser Ala Ser Arg Arg Val Gly Leu Ser Cys Ala
            260                 265                 270

Asn Cys Gln Thr Thr Thr Thr Thr Leu Trp Arg Arg Asn Ala Glu Gly
        275                 280                 285

Glu Pro Val Cys Asn Ala Cys Gly Leu Tyr Met Lys Leu His Gly Val
    290                 295                 300

Pro Arg Pro Leu Ala Met Arg Lys Glu Gly Ile Gln Thr Arg Lys Arg
305                 310                 315                 320

Lys Pro Lys Asn Leu Asn Lys Ser Lys Thr Pro Ala Ala Pro Ser Gly
                325                 330                 335

Ser Glu Ser Leu Pro Pro Ala Ser Gly Ala Ser Ser Asn Ser Ser Asn
            340                 345                 350

Ala Thr Thr Ser Ser Ser Glu Glu Met Arg Pro Ile Lys Thr Glu Pro
        355                 360                 365

Gly Leu Ser Ser His Tyr Gly His Ser Ser Ser Val Ser Gln Thr Phe
    370                 375                 380

Ser Val Ser Ala Met Ser Gly His Gly Pro Ser Ile His Pro Val Leu
385                 390                 395                 400

Ser Ala Leu Lys Leu Ser Pro Gln Gly Tyr Ala Ser Pro Val Ser Gln
                405                 410                 415

Ser Pro Gln Thr Ser Ser Lys Gln Asp Ser Trp Asn Ser Leu Val Leu
            420                 425                 430

Ala Asp Ser His Gly Asp Ile Ile Thr Ala
        435                 440
```

<210> 28
<211> 393
<212> PRT
<213> Homo sapiens

<400> 28

```
Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln
1               5                  10                  15


Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu Asn Asn Val Leu
            20                  25                  30


Ser Pro Leu Pro Ser Gln Ala Met Asp Asp Leu Met Leu Ser Pro Asp
        35                  40                  45


Asp Ile Glu Gln Trp Phe Thr Glu Asp Pro Gly Pro Asp Glu Ala Pro
    50                  55                  60


Arg Met Pro Glu Ala Ala Pro Pro Val Ala Pro Ala Pro Ala Ala Pro
65                  70                  75                  80


Thr Pro Ala Ala Pro Ala Pro Ala Pro Ser Trp Pro Leu Ser Ser Ser
                85                  90                  95


Val Pro Ser Gln Lys Thr Tyr Gln Gly Ser Tyr Gly Phe Arg Leu Gly
            100                 105                 110


Phe Leu His Ser Gly Thr Ala Lys Ser Val Thr Cys Thr Tyr Ser Pro
        115                 120                 125


Ala Leu Asn Lys Met Phe Cys Gln Leu Ala Lys Thr Cys Pro Val Gln
    130                 135                 140


Leu Trp Val Asp Ser Thr Pro Pro Pro Gly Thr Arg Val Arg Ala Met
145                 150                 155                 160


Ala Ile Tyr Lys Gln Ser Gln His Met Thr Glu Val Val Arg Arg Cys
```

```
                165                 170                 175

Pro His His Glu Arg Cys Ser Asp Ser Asp Gly Leu Ala Pro Pro Gln
            180                 185                 190

His Leu Ile Arg Val Glu Gly Asn Leu Arg Val Glu Tyr Leu Asp Asp
        195                 200                 205

Arg Asn Thr Phe Arg His Ser Val Val Val Pro Tyr Glu Pro Pro Glu
    210                 215                 220

Val Gly Ser Asp Cys Thr Thr Ile His Tyr Asn Tyr Met Cys Asn Ser
225                 230                 235                 240

Ser Cys Met Gly Gly Met Asn Arg Arg Pro Ile Leu Thr Ile Ile Thr
                245                 250                 255

Leu Glu Asp Ser Ser Gly Asn Leu Leu Gly Arg Asn Ser Phe Glu Val
            260                 265                 270

Arg Val Cys Ala Cys Pro Gly Arg Asp Arg Arg Thr Glu Glu Glu Asn
        275                 280                 285

Leu Arg Lys Lys Gly Glu Pro His His Glu Leu Pro Pro Gly Ser Thr
    290                 295                 300

Lys Arg Ala Leu Pro Asn Asn Thr Ser Ser Ser Pro Gln Pro Lys Lys
305                 310                 315                 320

Lys Pro Leu Asp Gly Glu Tyr Phe Thr Leu Gln Ile Arg Gly Arg Glu
                325                 330                 335

Arg Phe Glu Met Phe Arg Glu Leu Asn Glu Ala Leu Glu Leu Lys Asp
            340                 345                 350

Ala Gln Ala Gly Lys Glu Pro Gly Gly Ser Arg Ala His Ser Ser His
```

355 360 365

Leu Lys Ser Lys Lys Gly Gln Ser Thr Ser Arg His Lys Lys Leu Met
370 375 380

Phe Lys Thr Glu Gly Pro Asp Ser Asp
385 390

<210> 29
<211> 785
<212> PRT
<213> Homo sapiens

<400> 29

Met Ser Asp Gln Asp His Ser Met Asp Glu Met Thr Ala Val Val Lys
1 5 10 15

Ile Glu Lys Gly Val Gly Gly Asn Asn Gly Gly Asn Gly Asn Gly Gly
20 25 30

Gly Ala Phe Ser Gln Ala Arg Ser Ser Ser Thr Gly Ser Ser Ser Ser
35 40 45

Thr Gly Gly Gly Gly Gln Glu Ser Gln Pro Ser Pro Leu Ala Leu Leu
50 55 60

Ala Ala Thr Cys Ser Arg Ile Glu Ser Pro Asn Glu Asn Ser Asn Asn
65 70 75 80

Ser Gln Gly Pro Ser Gln Ser Gly Gly Thr Gly Glu Leu Asp Leu Thr
85 90 95

Ala Thr Gln Leu Ser Gln Gly Ala Asn Gly Trp Gln Ile Ile Ser Ser
100 105 110

Ser Ser Gly Ala Thr Pro Thr Ser Lys Glu Gln Ser Gly Ser Ser Thr
115 120 125

```
Asn Gly Ser Asn Gly Ser Glu Ser Ser Lys Asn Arg Thr Val Ser Gly
    130                 135                 140

Gly Gln Tyr Val Val Ala Ala Ala Pro Asn Leu Gln Asn Gln Gln Val
145                 150                 155                 160

Leu Thr Gly Leu Pro Gly Val Met Pro Asn Ile Gln Tyr Gln Val Ile
                165                 170                 175

Pro Gln Phe Gln Thr Val Asp Gly Gln Gln Leu Gln Phe Ala Ala Thr
            180                 185                 190

Gly Ala Gln Val Gln Gln Asp Gly Ser Gly Gln Ile Gln Ile Ile Pro
        195                 200                 205

Gly Ala Asn Gln Gln Ile Ile Thr Asn Arg Gly Ser Gly Gly Asn Ile
    210                 215                 220

Ile Ala Ala Met Pro Asn Leu Leu Gln Gln Ala Val Pro Leu Gln Gly
225                 230                 235                 240

Leu Ala Asn Asn Val Leu Ser Gly Gln Thr Gln Tyr Val Thr Asn Val
                245                 250                 255

Pro Val Ala Leu Asn Gly Asn Ile Thr Leu Leu Pro Val Asn Ser Val
            260                 265                 270

Ser Ala Ala Thr Leu Thr Pro Ser Ser Gln Ala Val Thr Ile Ser Ser
        275                 280                 285

Ser Gly Ser Gln Glu Ser Gly Ser Gln Pro Val Thr Ser Gly Thr Thr
    290                 295                 300

Ile Ser Ser Ala Ser Leu Val Ser Ser Gln Ala Ser Ser Ser Ser Phe
305                 310                 315                 320
```

```
Phe Thr Asn Ala Asn Ser Tyr Ser Thr Thr Thr Thr Ser Asn Met
                325                 330                 335

Gly Ile Met Asn Phe Thr Thr Ser Gly Ser Ser Gly Thr Asn Ser Gln
            340                 345                 350

Gly Gln Thr Pro Gln Arg Val Ser Gly Leu Gln Gly Ser Asp Ala Leu
        355                 360                 365

Asn Ile Gln Gln Asn Gln Thr Ser Gly Gly Ser Leu Gln Ala Gly Gln
    370                 375                 380

Gln Lys Glu Gly Glu Gln Asn Gln Gln Thr Gln Gln Gln Gln Ile Leu
385                 390                 395                 400

Ile Gln Pro Gln Leu Val Gln Gly Gly Gln Ala Leu Gln Ala Leu Gln
                405                 410                 415

Ala Ala Pro Leu Ser Gly Gln Thr Phe Thr Thr Gln Ala Ile Ser Gln
            420                 425                 430

Glu Thr Leu Gln Asn Leu Gln Leu Gln Ala Val Pro Asn Ser Gly Pro
        435                 440                 445

Ile Ile Ile Arg Thr Pro Thr Val Gly Pro Asn Gly Gln Val Ser Trp
    450                 455                 460

Gln Thr Leu Gln Leu Gln Asn Leu Gln Val Gln Asn Pro Gln Ala Gln
465                 470                 475                 480

Thr Ile Thr Leu Ala Pro Met Gln Gly Val Ser Leu Gly Gln Thr Ser
                485                 490                 495

Ser Ser Asn Thr Thr Leu Thr Pro Ile Ala Ser Ala Ala Ser Ile Pro
            500                 505                 510
```

```
Ala Gly Thr Val Thr Val Asn Ala Ala Gln Leu Ser Ser Met Pro Gly
        515                 520                 525

Leu Gln Thr Ile Asn Leu Ser Ala Leu Gly Thr Ser Gly Ile Gln Val
    530                 535                 540

His Pro Ile Gln Gly Leu Pro Leu Ala Ile Ala Asn Ala Pro Gly Asp
545                 550                 555                 560

His Gly Ala Gln Leu Gly Leu His Gly Ala Gly Gly Asp Gly Ile His
                565                 570                 575

Asp Asp Thr Ala Gly Gly Glu Glu Gly Glu Asn Ser Pro Asp Ala Gln
            580                 585                 590

Pro Gln Ala Gly Arg Arg Thr Arg Arg Glu Ala Cys Thr Cys Pro Tyr
        595                 600                 605

Cys Lys Asp Ser Glu Gly Arg Gly Ser Gly Asp Pro Gly Lys Lys Lys
    610                 615                 620

Gln His Ile Cys His Ile Gln Gly Cys Gly Lys Val Tyr Gly Lys Thr
625                 630                 635                 640

Ser His Leu Arg Ala His Leu Arg Trp His Thr Gly Glu Arg Pro Phe
                645                 650                 655

Met Cys Thr Trp Ser Tyr Cys Gly Lys Arg Phe Thr Arg Ser Asp Glu
            660                 665                 670

Leu Gln Arg His Lys Arg Thr His Thr Gly Glu Lys Lys Phe Ala Cys
        675                 680                 685

Pro Glu Cys Pro Lys Arg Phe Met Arg Ser Asp His Leu Ser Lys His
    690                 695                 700
```

```
Ile Lys Thr His Gln Asn Lys Lys Gly Gly Pro Gly Val Ala Leu Ser
705                 710                 715                 720


Val Gly Thr Leu Pro Leu Asp Ser Gly Ala Gly Ser Glu Gly Ser Gly
                725                 730                 735


Thr Ala Thr Pro Ser Ala Leu Ile Thr Thr Asn Met Val Ala Met Glu
            740                 745                 750


Ala Ile Cys Pro Glu Gly Ile Ala Arg Leu Ala Asn Ser Gly Ile Asn
        755                 760                 765


Val Met Gln Val Ala Asp Leu Gln Ser Ile Asn Ile Ser Gly Asn Gly
    770                 775                 780


Phe
785


<210>  30
<211>  473
<212>  PRT
<213>  Homo sapiens

<400>  30

Met Gly Arg Lys Lys Ile Gln Ile Thr Arg Ile Met Asp Glu Arg Asn
1               5                   10                  15


Arg Gln Val Thr Phe Thr Lys Arg Lys Phe Gly Leu Met Lys Lys Ala
            20                  25                  30


Tyr Glu Leu Ser Val Leu Cys Asp Cys Glu Ile Ala Leu Ile Ile Phe
        35                  40                  45


Asn Ser Thr Asn Lys Leu Phe Gln Tyr Ala Ser Thr Asp Met Asp Lys
    50                  55                  60
```

```
Val Leu Leu Lys Tyr Thr Glu Tyr Asn Glu Pro His Glu Ser Arg Thr
65                  70                  75                  80

Asn Ser Asp Ile Val Glu Thr Leu Arg Lys Lys Gly Leu Asn Gly Cys
                85                  90                  95

Asp Ser Pro Asp Pro Asp Ala Asp Asp Ser Val Gly His Ser Pro Glu
            100                 105                 110

Ser Glu Asp Lys Tyr Arg Lys Ile Asn Glu Asp Ile Asp Leu Met Ile
        115                 120                 125

Ser Arg Gln Arg Leu Cys Ala Val Pro Pro Pro Asn Phe Glu Met Pro
    130                 135                 140

Val Ser Ile Pro Val Ser Ser His Asn Ser Leu Val Tyr Ser Asn Pro
145                 150                 155                 160

Val Ser Ser Leu Gly Asn Pro Asn Leu Leu Pro Leu Ala His Pro Ser
                165                 170                 175

Leu Gln Arg Asn Ser Met Ser Pro Gly Val Thr His Arg Pro Pro Ser
            180                 185                 190

Ala Gly Asn Thr Gly Gly Leu Met Gly Gly Asp Leu Thr Ser Gly Ala
        195                 200                 205

Gly Thr Ser Ala Gly Asn Gly Tyr Gly Asn Pro Arg Asn Ser Pro Gly
    210                 215                 220

Leu Leu Val Ser Pro Gly Asn Leu Asn Lys Asn Met Gln Ala Lys Ser
225                 230                 235                 240

Pro Pro Pro Met Asn Leu Gly Met Asn Asn Arg Lys Pro Asp Leu Arg
                245                 250                 255
```

```
Val Leu Ile Pro Pro Gly Ser Lys Asn Thr Met Pro Ser Val Ser Glu
            260                 265                 270


Asp Val Asp Leu Leu Leu Asn Gln Arg Ile Asn Asn Ser Gln Ser Ala
        275                 280                 285


Gln Ser Leu Ala Thr Pro Val Val Ser Val Ala Thr Pro Thr Leu Pro
    290                 295                 300


Gly Gln Gly Met Gly Gly Tyr Pro Ser Ala Ile Ser Thr Thr Tyr Gly
305                 310                 315                 320


Thr Glu Tyr Ser Leu Ser Ser Ala Asp Leu Ser Ser Leu Ser Gly Phe
                325                 330                 335


Asn Thr Ala Ser Ala Leu His Leu Gly Ser Val Thr Gly Trp Gln Gln
            340                 345                 350


Gln His Leu His Asn Met Pro Pro Ser Ala Leu Ser Gln Leu Gly Ala
        355                 360                 365


Cys Thr Ser Thr His Leu Ser Gln Ser Ser Asn Leu Ser Leu Pro Ser
    370                 375                 380


Thr Gln Ser Leu Asn Ile Lys Ser Glu Pro Val Ser Pro Pro Arg Asp
385                 390                 395                 400


Arg Thr Thr Thr Pro Ser Arg Tyr Pro Gln His Thr Arg His Glu Ala
                405                 410                 415


Gly Arg Ser Pro Val Asp Ser Leu Ser Ser Cys Ser Ser Ser Tyr Asp
            420                 425                 430


Gly Ser Asp Arg Glu Asp His Arg Asn Glu Phe His Ser Pro Ile Gly
        435                 440                 445
```

```
Leu Thr Arg Pro Ser Pro Asp Glu Arg Glu Ser Pro Ser Val Lys Arg
    450                 455                 460


Met Arg Leu Ser Glu Gly Trp Ala Thr
465                 470


<210>  31
<211>  353
<212>  PRT
<213>  Unknown

<220>
<223>  Human T-lymphotrophic virus

<400>  31

Met Ala His Phe Pro Gly Phe Gly Gln Ser Leu Leu Phe Gly Tyr Pro
1               5                   10                  15


Val Tyr Val Phe Gly Asp Cys Val Gln Gly Asp Trp Cys Pro Ile Ser
            20                  25                  30


Gly Gly Leu Cys Ser Ala Arg Leu His Arg His Ala Leu Leu Ala Thr
        35                  40                  45


Cys Pro Glu His Gln Ile Thr Trp Asp Pro Ile Asp Gly Arg Val Ile
    50                  55                  60


Gly Ser Ala Leu Gln Phe Leu Ile Pro Arg Leu Pro Ser Phe Pro Thr
65                  70                  75                  80


Gln Arg Thr Ser Lys Thr Leu Lys Val Leu Thr Pro Pro Ile Thr His
                85                  90                  95


Thr Thr Pro Asn Ile Pro Pro Ser Phe Leu Gln Ala Met Arg Lys Tyr
            100                 105                 110


Ser Pro Phe Arg Asn Gly Tyr Met Glu Pro Thr Leu Gly Gln His Leu
```

```
        115                 120                 125

Pro Thr Leu Ser Phe Pro Asp Pro Gly Leu Arg Pro Gln Asn Leu Tyr
    130                 135                 140

Thr Leu Trp Gly Gly Ser Val Val Cys Met Tyr Leu Tyr Gln Leu Ser
145                 150                 155                 160

Pro Pro Ile Thr Trp Pro Leu Leu Pro His Val Ile Phe Cys His Pro
                165                 170                 175

Gly Gln Leu Gly Ala Phe Leu Thr Asn Val Pro Tyr Lys Arg Ile Glu
            180                 185                 190

Glu Leu Leu Tyr Lys Ile Ser Leu Thr Thr Gly Ala Leu Ile Ile Leu
        195                 200                 205

Pro Glu Asp Cys Leu Pro Thr Thr Leu Phe Gln Pro Ala Arg Ala Pro
    210                 215                 220

Val Thr Leu Thr Ala Trp Gln Asn Gly Leu Leu Pro Phe His Ser Thr
225                 230                 235                 240

Leu Thr Thr Pro Gly Leu Ile Trp Thr Phe Thr Asp Gly Thr Pro Met
                245                 250                 255

Ile Ser Gly Pro Cys Pro Lys Asp Gly Gln Pro Ser Leu Val Leu Gln
            260                 265                 270

Ser Ser Ser Phe Ile Phe His Lys Phe Gln Thr Lys Ala Tyr His Pro
        275                 280                 285

Ser Phe Leu Leu Ser His Gly Leu Ile Gln Tyr Ser Ser Phe His Ser
    290                 295                 300

Leu His Leu Leu Phe Glu Glu Tyr Thr Asn Ile Pro Ile Ser Leu Leu
```

305 310 315 320

Phe Asn Glu Lys Glu Ala Asp Asp Asn Asp His Glu Pro Gln Ile Ser
325 330 335

Pro Gly Gly Leu Glu Pro Pro Ser Glu Lys His Phe Arg Glu Thr Glu
340 345 350

Val

<210> 32
<211> 505
<212> PRT
<213> Homo sapiens

<400> 32

Met Gly Glu Thr Leu Gly Asp Ser Pro Ile Asp Pro Glu Ser Asp Ser
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Phe Thr Asp Thr Leu Ser Ala Asn Ile Ser Gln Glu Met Thr Met Val
20 25 30

Asp Thr Glu Met Pro Phe Trp Pro Thr Asn Phe Gly Ile Ser Ser Val
35 40 45

Asp Leu Ser Val Met Glu Asp His Ser His Ser Phe Asp Ile Lys Pro
50 55 60

Phe Thr Thr Val Asp Phe Ser Ser Ile Ser Thr Pro His Tyr Glu Asp
65 70 75 80

Ile Pro Phe Thr Arg Thr Asp Pro Val Val Ala Asp Tyr Lys Tyr Asp
85 90 95

Leu Lys Leu Gln Glu Tyr Gln Ser Ala Ile Lys Val Glu Pro Ala Ser
100 105 110

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Pro Pro Tyr Tyr Ser Glu Lys Thr Gln Leu Tyr Asn Lys Pro His Glu
        115                 120                 125

Glu Pro Ser Asn Ser Leu Met Ala Ile Glu Cys Arg Val Cys Gly Asp
    130                 135                 140

Lys Ala Ser Gly Phe His Tyr Gly Val His Ala Cys Glu Gly Cys Lys
145                 150                 155                 160

Gly Phe Phe Arg Arg Thr Ile Arg Leu Lys Leu Ile Tyr Asp Arg Cys
                165                 170                 175

Asp Leu Asn Cys Arg Ile His Lys Lys Ser Arg Asn Lys Cys Gln Tyr
            180                 185                 190

Cys Arg Phe Gln Lys Cys Leu Ala Val Gly Met Ser His Asn Ala Ile
        195                 200                 205

Arg Phe Gly Arg Met Pro Gln Ala Glu Lys Glu Lys Leu Leu Ala Glu
    210                 215                 220

Ile Ser Ser Asp Ile Asp Gln Leu Asn Pro Glu Ser Ala Asp Leu Arg
225                 230                 235                 240

Ala Leu Ala Lys His Leu Tyr Asp Ser Tyr Ile Lys Ser Phe Pro Leu
                245                 250                 255

Thr Lys Ala Lys Ala Arg Ala Ile Leu Thr Gly Lys Thr Thr Asp Lys
            260                 265                 270

Ser Pro Phe Val Ile Tyr Asp Met Asn Ser Leu Met Met Gly Glu Asp
        275                 280                 285

Lys Ile Lys Phe Lys His Ile Thr Pro Leu Gln Glu Gln Ser Lys Glu
    290                 295                 300
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Val Ala Ile Arg Ile Phe Gln Gly Cys Gln Phe Arg Ser Val Glu Ala
305                 310                 315                 320

Val Gln Glu Ile Thr Glu Tyr Ala Lys Ser Ile Pro Gly Phe Val Asn
                325                 330                 335

Leu Asp Leu Asn Asp Gln Val Thr Leu Leu Lys Tyr Gly Val His Glu
            340                 345                 350

Ile Ile Tyr Thr Met Leu Ala Ser Leu Met Asn Lys Asp Gly Val Leu
        355                 360                 365

Ile Ser Glu Gly Gln Gly Phe Met Thr Arg Glu Phe Leu Lys Ser Leu
    370                 375                 380

Arg Lys Pro Phe Gly Asp Phe Met Glu Pro Lys Phe Glu Phe Ala Val
385                 390                 395                 400

Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Asp Leu Ala Ile Phe Ile
                405                 410                 415

Ala Val Ile Ile Leu Ser Gly Asp Arg Pro Gly Leu Leu Asn Val Lys
            420                 425                 430

Pro Ile Glu Asp Ile Gln Asp Asn Leu Leu Gln Ala Leu Glu Leu Gln
        435                 440                 445

Leu Lys Leu Asn His Pro Glu Ser Ser Gln Leu Phe Ala Lys Leu Leu
    450                 455                 460

Gln Lys Met Thr Asp Leu Arg Gln Ile Val Thr Glu His Val Gln Leu
465                 470                 475                 480

Leu Gln Val Ile Lys Lys Thr Glu Thr Asp Met Ser Leu His Pro Leu
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Leu Gln Glu Ile Tyr Lys Asp Leu Tyr
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<400> 33

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Asp Asp Asp Gly Leu Pro His Gly Phe Cys Thr Val Thr Tyr Ser Ser
20 25 30

Thr Asp Arg Phe Glu Gly Asn Phe Val His Gly Glu Lys Asn Gly Arg
35 40 45

Gly Lys Phe Phe Phe Phe Asp Gly Ser Thr Leu Glu Gly Tyr Tyr Val
50 55 60

Asp Asp Ala Leu Gln Gly Gln Gly Val Tyr Thr Tyr Glu Asp Gly Gly
65 70 75 80

Val Leu Gln Gly Thr Tyr Val Asp Gly Glu Leu Asn Gly Pro Ala Gln
85 90 95

Glu Tyr Asp Thr Asp Gly Arg Leu Ile Phe Lys Gly Gln Tyr Lys Asp
100 105 110

Asn Ile Arg His Gly Val Cys Trp Ile Tyr Tyr Pro Asp Gly Gly Ser
115 120 125

Leu Val Gly Glu Val Asn Glu Asp Gly Glu Met Thr Gly Glu Lys Ile
130 135 140

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Ala Tyr Val Tyr Pro Asp Glu Arg Thr Ala Leu Tyr Gly Lys Phe Ile
145                 150                 155                 160


Asp Gly Glu Met Ile Glu Gly Lys Leu Ala Thr Leu Met Ser Thr Glu
                165                 170                 175


Glu Gly Arg Pro His Phe Glu Leu Met Pro Gly Asn Ser Val Tyr His
            180                 185                 190


Phe Asp Lys Ser Thr Ser Ser Cys Ile Ser Thr Asn Ala Leu Leu Pro
        195                 200                 205


Asp Pro Tyr Glu Ser Glu Arg Val Tyr Val Ala Glu Ser Leu Ile Ser
    210                 215                 220


Ser Ala Gly Glu Gly Leu Phe Ser Lys Val Ala Val Gly Pro Asn Thr
225                 230                 235                 240


Val Met Ser Phe Tyr Asn Gly Val Arg Ile Thr His Gln Glu Val Asp
                245                 250                 255


Ser Arg Asp Trp Ala Leu Asn Gly Asn Thr Leu Ser Leu Asp Glu Glu
            260                 265                 270


Thr Val Ile Asp Val Pro Glu Pro Tyr Asn His Val Ser Lys Tyr Cys
        275                 280                 285


Ala Ser Leu Gly His Lys Ala Asn His Ser Phe Thr Pro Asn Cys Ile
    290                 295                 300


Tyr Asp Met Phe Val His Pro Arg Phe Gly Pro Ile Lys Cys Ile Arg
305                 310                 315                 320


Thr Leu Arg Ala Val Glu Ala Asp Glu Glu Leu Thr Val Ala Tyr Gly
                325                 330                 335
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Tyr Asp His Ser Pro Pro Gly Lys Ser Gly Pro Glu Ala Pro Glu Trp
340 345 350

Tyr Gln Val Glu Leu Lys Ala Phe Gln Ala Thr Gln Gln Lys
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<220>
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gggaagaaag ccccacttgg 20

<210> 36
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<220>
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<400> 36
gcccagtcgc gtgggggggg 20

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<213> Artificial Sequence

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<213> Mus musculus

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<400> 40
cgagccgcga gcccagtcgc g 21

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ctgtgaaagg aatacaattc a 21

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gatgtctcag aaccactgaa t 21

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<210> 74
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<213> Homo sapiens

<400> 74
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<210> 75
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<400> 75
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<210> 76
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<400> 76
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