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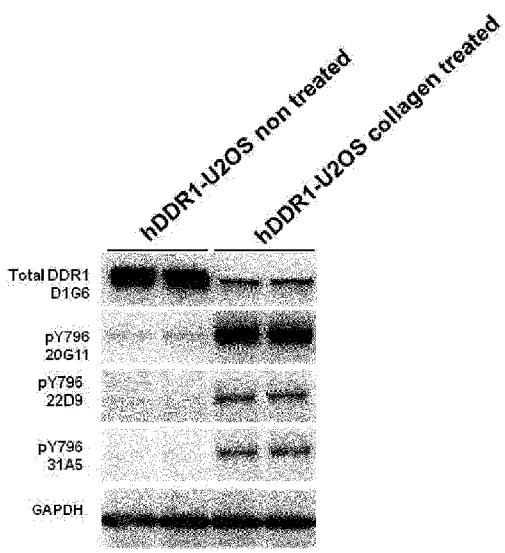

Figure 2

(57) Abstract: The present invention relates to an antibody as characterized in the appended claims, wherein the antibody specifically binds to the discoidin domain receptor 1 (DDR1) which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17. The present invention further relates to nucleic acid molecules encoding the light chain variable region or the heavy chain variable region of the antibody of the invention, as well as vectors comprising said nucleic acid molecules. The invention further relates to a host cell or non-human host comprising the vector(s) of the invention, as well as to a method for the production of an antibody according to the invention comprising culturing the host cell of the invention under suitable conditions and isolating the antibody produced. Furthermore, the present invention relates to an antibody obtainable by the method of the invention, to a composition comprising at least one of the antibody of the invention, the nucleic acid molecule of the invention, the vector of the invention, the host cell of the invention or the antibody produced by the method of the invention. The present invention also relates to the use of the antibody of the invention for determining phosphorylation of DDR1 at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17 as well as to a method of determining phosphorylation of DDR1 at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17.

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Novel anti-pY$_{796}$-DDR1 antibodies

The present invention relates to an antibody as characterized in the appended claims, wherein the antibody specifically binds to the discoidin domain receptor 1 (DDR1) which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17. The present invention further relates to nucleic acid molecules encoding the light chain variable region or the heavy chain variable region of the antibody of the invention, as well as vectors comprising said nucleic acid molecules. The invention further relates to a host cell or non-human host comprising the vector(s) of the invention, as well as to a method for the production of an antibody according to the invention comprising culturing the host cell of the invention under suitable conditions and isolating the antibody produced. Furthermore, the present invention relates to an antibody obtainable by the method of the invention, to a composition comprising at least one of the antibody of the invention, the nucleic acid molecule of the invention, the vector of the invention, the host cell of the invention or the antibody produced by the method of the invention. The present invention also relates to the use of the antibody of the invention for determining phosphorylation of DDR1 at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17 as well as to a method of determining phosphorylation of DDR1 at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17.

In this specification, a number of documents including patent applications and manufacturer's manuals are cited. The disclosure of these documents, while not considered relevant for the patentability of this invention, is herewith incorporated by reference in its entirety. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

The discoidin domain receptor DDR1 is a receptor tyrosine kinase (RTK) and a non-integrin collagen receptor that binds a number of different collagen types and plays important roles in

e.g. embryo development (for a review see Leiting B. (2014), International Review of Cell and Molecular Biology, Volume 310: 39-87 and Borza CM and Pozzi A (2014), Matrix Biology 34 185–192). DDR-facilitated cellular functions include cell migration, cell survival, proliferation, and differentiation, as well as remodeling of extracellular matrices (ECM) expression and activity via the control of matrix metalloproteinase (MMP).

DDR1 is mainly expressed in epithelial cells, but is also found in cells of the immune system, such as stimulated peripheral blood mononuclear cells and on activated T cells. DDR1 has further been shown to be able to mediate cell migration of monocytic cells and T cells in three-dimensional (3D) collagen matrices. Thus, DDR1 appears to be an important player also in immune responses, which depend on the effective migration of activated leukocytes into infectious or inflammatory tissue sites.

The human DDR1 gene maps to chromosome 6 (6p21.3) at the major histocompatibility complex locus between the HLA-E and HLA-C genes. The DDR1 gene spans 24 kb and comprises 17 exons. The extracellular domain is encoded by exons 1 to 8, the transmembrane domain by exon 9. Exons 10 to 12 encode the cytosolic juxtamembrane (JM) domain, with the remaining exons predominantly coding for the catalytic domain.

DDR1 is a typical receptor tyrosine kinase in that it is a single span type I transmembrane protein with a C-terminal tyrosine kinase domain in the cytoplasmic region. Receptor tyrosine kinases transmit signals into cells by providing docking sites for effector molecules in the form of phosphorylated cytoplasmic tyrosines, a result of ligand-induced kinase dimerization and subsequent receptor autophosphorylation. However, the DDRs (i.e. DDR1 and DDR2) are unusual RTKs in that they form ligand-independent stable dimers that are non-covalently linked. Therefore, the paradigm of ligand-induced receptor dimerization does not apply to the DDRs. Nonetheless, DDR1 undergoes ligand-induced autophosphorylation like all receptor tyrosine kinases. However, little is known about the molecular mechanism of DDR transmembrane signaling and the translation of collagen binding to an activation of the kinase domain.

Five different DDR1 isoforms have been identified, which result from alternative splicing of exons 10 to 14 of the DDR1 gene. Tyrosine at position 796 in the DDR1 sequence is present in isoforms 1, 2, 4 and 5 and is one tyrosine in the DDR1 sequence that becomes phosphorylated

upon ligand binding. This phosphorylation site provides a docking site for the down-stream effectors SH2-containing transforming protein A (ShcA), SH2-containing inositol polyphosphate 5-phosphatase 1/2 (SHIP1/2) and SH2-containing protein tyrosine phosphatase 2 (SHP-2). However, the signaling pathways activated by DDRs are not completely understood and may differ depending on the context and cell-type (Borza CM and Pozzi A (2014), Matrix Biology 34 185–192).

Dysregulated DDR function is associated with the progression of various human diseases, including fibrosis, atherosclerosis, arthritis, and cancer. For example, artherosclerosis is a disease characterized by thickened neointimal lesions in the vessel wall to which smooth muscle cells (SMCs) contribute by increased proliferation and migration, as well as MMP and ECM synthesis. Mice lacking DDR1 were found to be protected from this intimal thickening after mechanical carotid injury and showed decreased SMC proliferation, MMP production, and ECM synthesis. DDR1 has also been shown to be a regulator of kidney disease. Using a mouse model, it has been shown that Dddr1-negative mice are protected against hypertension-induced kidney disease and that DDR1 mediates both inflammation and fibrosis in this pathology. A similar pathophysiological role for DDR1 was also found in another kidney disorder, Alport syndrome. Furthermore, DDR1-negative mice were also protected from crescentic glomerulonephritis and obstructive nephropathy. In both pathologies, DDR1 mediated inflammatory responses and fibrosis. Similarly, mice devoid of DDR1 were protected from bleomycin-induced lung injury, indicating that DDR1 may be important in modulating idiopathic pulmonary fibrosis, which is characterized by persistent epithelial injury. In addition, DDRs play a key role in cancer progression, in part by regulating the interaction of cancer cells with collagens. DDRs are overexpressed in a large number of different types of cancer, ranging from lung, breast, brain, esophagus, head and neck, liver, and prostate cancers to lymphomas and leukemias. For example, DDR1 activation in breast and colon carcinoma cell lines was found to trigger pro-survival signals. Moreover, DDR1 can confer resistance to chemotherapy in breast cancer and lymphoma cell lines. However, the molecular mechanisms underlying the roles of the DDRs in various steps of cancer progression are largely undefined (see e.g. Leiting B., International Review of Cell and Molecular Biology, Volume 310: 39-87 and Borza CM and Pozzi A (2014), Matrix Biology 34 185–192).

Despite the fact that DDR1 intracellular signaling and its role in a range of pathologies is currently investigated by various research groups, there is still a need to shed light on the

mechanisms of DDR1 mediated signaling events, on factors that influence said signaling pathways and in particular on the role of DDR1 in disease pathologies. To this end, tools are required that enable a more efficient and accurate monitoring of the activation state of the DDR1 receptor upon stimulation.

This need is addressed by the present invention by providing the embodiments as defined in the claims.

Accordingly, the present invention relates to an antibody that specifically binds to the discoidin domain receptor 1 (DDR1) which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17, wherein the antibody is characterized in that the CDRs comprise the following amino acid sequences or a variant thereof that differs in at most one amino acid substitution:

(i) in the light chain variable domain a CDR1 comprising the amino acid sequence of SEQ ID NO:1, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4, and in the heavy chain variable domain a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7; or

(ii) in the light chain variable domain a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4, and in the heavy chain variable domain a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7.

The overall structure of antibodies is well known in the art and comprises of two heavy chains and two light chains, connected by disulfide bonds. The heavy chains and the light chains each consist of one constant domain and one variable domain. Binding specificity to an antigen is provided by the variable domains of the light and heavy chains that form the antibody. More specifically, the parts of antibodies that determine their specificity and make contact with a specific ligand are referred to as the complementarity determining regions (CDRs). The CDRs are the most variable part of the molecule and contribute to the diversity of these molecules. There are three CDR regions CDR1, CDR2 and CDR3 in each variable domain, embedded into

four framework regions (FW). As used herein, CDR-HC (or CDR(HC)) depicts a CDR region of a variable heavy chain and CDR-LC (or CDR(LC)) relates to a CDR region of a variable light chain. Similarly, FW-HC (or FW(HC)) depicts a framework region of a variable heavy chain and FW-LC (or FW(LC)) relates to a framework region of a variable light chain.

The term "comprising", as used in accordance with the present invention, denotes that further sequences/components can be included in addition to the specifically recited sequences and/or components. However, this term also encompasses that the claimed subject-matter consists of exactly the recited sequences and/or components.

In those embodiments where the antibody of the invention includes more than the recited amino acid sequence, additional amino acids extend can be present at either the N-terminal end, or the C-terminal end, or both. Additional sequences can include e.g. sequences introduced e.g. for purification or detection, as discussed in detail herein below. Furthermore, where individual sequences "comprise" the recited sequence, they also can include additional amino acids at either the N-terminal end, or the C-terminal end, or both.

In accordance with the present invention, the antibody specifically binds to DDR1 which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17. The sequence represented in SEQ ID NO:17 represents isoform 1 of DDR1. Accordingly, this antibody is also referred to herein as an anti-pY_{796}-DDR1 antibody. Furthermore, the amino acid sequence of DDR1 comprising a phosphorylated tyrosine at position 796 of the sequence represented in SEQ ID NO:17 is also referred to herein as pY_{796}-DDR1. It will be appreciated that also in the cases where the antibody of the invention comprises additional amino acids, as detailed above, said antibody necessarily has to specifically bind to pY_{796}-DDR1.

The term "specifically binds" (also referred to herein as "specifically interacts"), in accordance with the present invention, means that the antibody specifically binds only DDR1 when DDR1 is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17, but does not or essentially does not cross-react with a different protein, in particular a different protein of similar structure such as e.g. DDR2, or with DDR1 that is not phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17.

Corresponding methods for analyzing the specificity of an antibody are described e.g. in Harlow & Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, and in Harlow & Lane (1999) Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press. Non-limiting examples of suitable studies are e.g. binding studies, blocking and competition studies with structurally and/or functionally closely related molecules. These studies can be carried out by methods such as e.g. FACS analysis, flow cytometric titration analysis (FACS titration), surface plasmon resonance (SPR, e.g. with BIAcore®), isothermal titration calorimetry (ITC), fluorescence titration, or by radiolabeled ligand binding assays. Further methods include e.g. western blots, ELISA (including competition ELISA)-, RIA-, ECL-, IRMA-tests, as well as physiological assays, like cytotoxic assays. In one embodiment, specificity for DDR1 is determined by western blots using lysates of collagen stimulated cells, expressing high levels of phosphorylated DDR1. Unstimulated cell lysates can be used as negative control. To verify the specificity of the antibodies for the phosphorylation of the tyrosine in position 796 of DDR1 it is recommended to perform surface plasmon resonance (SPR, e.g. with BIAcore®) experiments with a peptide covering the amino acid sequence around said the tyrosine in position 796 of the DDR1 sequence, wherein said tyrosine carries a phosphorylation. As a negative control, a peptide without a phosphorylation but otherwise identical sequence should be used.

Cross-reactivity of a panel of antibodies under investigation can be tested, for example, by assessing binding of said panel of antibodies under conventional conditions to pY_{796}-DDR1 as well as to a number of more or less (structurally and/or functionally) closely related proteins, and to DDR1 that is not phosphorylated at the tyrosine in position 796. Only those molecules that bind to pY_{796}-DDR1 but do not or do not essentially bind to any of the other proteins or to DDR1 that is not phosphorylated at the tyrosine in position 796 are considered to bind specifically.

The term "a molecule that essentially does not cross-react", as used herein, refers to a molecule that binds to the target molecule (i.e. pY_{796}-DDR1) with at least 5-times higher affinity than to a different protein of similar structure and to DDR1 that is not phosphorylated at the tyrosine in position 796, more preferably at least 10-times higher affinity, such as e.g. at least 50-times higher affinity, more preferably at least 100-times higher affinity, such as e.g. at least 250-

times higher affinity. Even more preferably, it binds with at least 500-times higher affinity to pY_{796}-DDR1 than to a different protein of similar structure and to DDR1 that is not phosphorylated at the tyrosine in position 796, and most preferably with at least 1.000-times higher affinity.

In context of the present invention, the term “antibody” relates to full immunoglobulin molecules as well as to antigen binding fragments thereof, like, Fab, Fab’, $F(ab')_2$,, Fv. Furthermore, the term relates to modified and/or altered antibody molecules, as well as to recombinantly or synthetically generated/synthesized antibodies. The term “antibody” also comprises bifunctional antibodies, trifunctional antibodies, fully-human antibodies, chimeric antibodies, and antibody constructs, like single chain Fvs (scFv) or antibody-fusion proteins.

A “Fab fragment” as used herein is comprised of one light chain and the C_H1 and variable regions of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. A "Fab' fragment" contains one light chain and a portion of one heavy chain that contains the V_H domain and the C_H1 domain and also the region between the C_H1 and C_H2 domains, such that an interchain disulfide bond can be formed between the two heavy chains of two Fab' fragments to form a $F(ab')_2$ molecule. A "$F(ab')_2$ fragment" contains two light chains and two heavy chains containing a portion of the constant region between the C_H1 and C_H2 domains, such that an interchain disulfide bond is formed between the two heavy chains. A $F(ab')_2$ fragment thus is composed of two Fab' fragments that are held together by a disulfide bond between the two heavy chains.

Fab/c fragment contain both Fc and Fab determinants, wherein an "Fc" region contains two heavy chain fragments comprising the C_H2 and C_H3 domains of an antibody. The two heavy chain fragments are held together by two or more disulfide bonds and by hydrophobic interactions of the C_H3 domains.

The "Fv region" comprises the variable regions from both the heavy and light chains, but lacks the constant regions. “Single-chain Fvs” (also abbreviated as “scFv”) are antibody fragments that have, in the context of the present invention, the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. Techniques described for the

production of single chain antibodies are described, e.g., in Plückthun in The Pharmacology of Monoclonal Antibodies, Rosenburg and Moore eds. Springer-Verlag, N.Y. 113 (1994), 269-315.

The term “fully-human antibody” as used herein refers to an antibody which comprises human immunoglobulin protein sequences only. Nonetheless, a fully human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell or in a hybridoma derived from a mouse cell or it may contain rat carbohydrate chains if produced in a rat, in a rat cell, or in a hybridoma derived from a rat cell. Similarly, a fully human antibody may contain hamster carbohydrate chains if produced in a hamster, in a hamster cell, such as e.g. CHO cells, or in a hybridoma derived from a hamster cell. On the other hand, a “mouse antibody” or “murine antibody” is an antibody that comprises mouse (murine) immunoglobulin protein sequences only, while a “rat antibody” or a "rabbit antibody" is an antibody that comprises rat or rabbit immunoglobulin sequences, respectively, only. As with fully human antibodies, such murine, rat or rabbit antibodies may contain carbohydrate chains from other species, if produced in such an animal or a cell of such an animal. For example, the antibodies may contain hamster carbohydrate chains if produced in a hamster cell, such as e.g. CHO cells, or in a hybridoma derived from a hamster cell. Fully-human antibodies can be produced, for example, by phage display which is a widely used screening technology which enables production and screening of fully human antibodies. Also phage antibodies can be used in context of this invention. Phage display methods are described, for example, in US 5,403,484, US 5,969,108 and US 5,885,793. Another technology which enables development of fully-human antibodies involves a modification of mouse hybridoma technology. Mice are made transgenic to contain the human immunoglobulin locus in exchange for their own mouse genes (see, for example, US 5,877,397).

The term “chimeric antibodies” refers to antibodies that comprise a variable region of a human or non-human species fused or chimerized to an antibody region (*e.g.*, constant region) from another species, either human or non-human (*e.g.*, mouse, horse, rabbit, dog, cow, chicken).

As mentioned above, the term "antibody" also encompasses antibody constructs, such as antibody-fusion proteins, wherein the antibody comprises (an) additional domain(s), e.g. for the isolation and/or preparation of recombinantly produced constructs, in addition to the domains defined herein by specific amino acid sequences.

The antibody of the present invention can be produced such that it is a recombinant antibody, for example a recombinant human antibody, a heterologous antibody or a hetero-hybrid antibody. The term "recombinant (human) antibody" includes all (human sequence) antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes, antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Recombinant human antibodies have variable and constant regions (if present) derived from human germline immunoglobulin sequences. Such antibodies can, however, be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

A "heterologous antibody" is defined in relation to the transgenic non-human organism producing such an antibody. This term refers to an antibody having an amino acid sequence or an encoding nucleic acid sequence corresponding to that found in an organism that is not the transgenic non-human animal, and generally from a species other than that of the transgenic non-human animal.

The term "hetero-hybrid antibody" refers to an antibody having light and heavy chains that originate from different organisms. For example, an antibody having a human heavy chain associated with a murine light chain is a hetero-hybrid antibody. Examples of hetero-hybrid antibodies include chimeric and humanized antibodies.

The antibody in accordance with the present invention comprises one of the four recited combinations (i) to (iv) of light chain CRDs and heavy chain CRDs. The surrounding framework sequence of the respective variable domain into which the CDRs are incorporated can be chosen by the skilled person without further ado. For example, the framework sequences described further below or the specific framework sequence employed in the appended examples can be used.

In accordance with the present invention, the CDRs can comprise the specifically recited sequence or can differ therefrom in at most one amino acid substitution. As such, one amino acid in each of the CDRs can be replaced by a different amino acid. It will be appreciated that also encompassed is that an amino acid substitution is present in some, but not all CDRs of one chain or of one antibody.

The term "substitution", in accordance with the present invention, refers to the replacement of an amino acid with another amino acid. Thus, the total number of amino acids remains the same. The deletion of an amino acid at a certain position and the introduction of one (or more) amino acid(s) at a different position is explicitly not encompassed by the term "substitution". Substitutions, in accordance with the present invention, can be conservative amino acid substitutions or non-conservative amino acid substitutions. The term "conservative amino acid substitution" is well known in the art and refers to the replacement of an amino acid with a different amino acid having similar structural and/or chemical properties. Such similarities include e.g. a similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Non-conservative amino acid substitutions can be introduced in order to introduce new reactive groups, for example, for the conjugation to other compounds, such as polyethylene glycol (PEG), hydroxyethyl starch (HES), biotin, peptides or proteins, or for the formation of non-naturally occurring intermolecular disulphide linkages. To this end, cysteine can be introduced into the amino acid sequence. The thiol moiety thus generated can then be used for the conjugation to other compounds, for example, in order to increase the serum half-life of the respective anti-pY_{796}-DDR1 antibody. Accordingly, in one embodiment of the present invention where the substitution is a non-conservative amino acid substitution, it is a substitution that introduces a cysteine. In one embodiment, the substitution in any (or all) of the CDRs is a conservative amino acid substitution. It will be appreciated that also an antibody having such substituted amino acids in one or more of the CDRs necessarily has to be an antibody that specifically binds to the discoidin domain receptor 1 (DDR1) which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID

NO:17, as defined herein above.

In one embodiment, the CDRs comprise the sequence specifically recited above, i.e. without any amino acid variation.

In accordance with the present invention, novel anti-pY_{796}-DDR1 antibodies are provided that enable the specific and efficient monitoring of DDR1 receptor activation. As discussed herein above, DDR1 has been shown to play important roles in various diseases and, accordingly, monitoring the activation of DDR1 and its signaling activity is of utmost interest for understanding the involvement of DDR1 in said diseases.

As is shown in the appended examples, the anti-pY_{796}-DDR1 antibodies of the present invention provide a superior binding efficiency and specificity. Antibody-binding to pY_{796}-DDR1 was determined in five different assays, including immunohistochemical staining of collagen stimulated (positive control) and unstimulated formalin-fixed paraffin embedded cells; western blots with lysates from collagen stimulated (positive control) and unstimulated cells; ELISA and Biacore with phosphorylated and non-phosphorylated peptides; as well as a peptide array with spotted phosphorylated and non-phosphorylated peptides spanning amino acid sequences of related phosphorylation sites from other receptor tyrosine kinases. In these assays, the anti-pY_{796}-DDR1 antibodies according to the present invention showed a surprising capability to bind to phosphorylated tyrosine at position 796 of the DDR1 sequence represented in SEQ ID NO:17, whereas DDR1 that is not phosphorylated at this position was not bound.

Furthermore, the present invention also relates to the antibody of the invention, wherein the antibody comprises:

a light chain variable domain consisting of framework regions (FW) and CDRs as represented in formula I:

FW(LC)1 – CDR(LC)1 – FW(LC)2 – CDR(LC)2 – FW(LC)3 – CDR(LC)3 – FW(LC)4 (formula I)

and a heavy chain variable domain consisting of FWs and CDRs as represented in formula II:

FW(HC)1 – CDR(HC)1 – FW(HC)2 – CDR(HC)2 – FW(HC)3 – CDR(HC)3 – FW(HC)4 (formula II),

wherein the FWs comprise the following amino acid sequences or a variant thereof that is at least 85% identical thereto and wherein the CDRs comprise the following amino acid sequences or a variant thereof that differs in at most one amino acid substitution:

(i)	FW(LC)1	the amino acid sequence of SEQ ID NO:8;
	CDR(LC)1	the amino acid sequence of SEQ ID NO:1;
	FW(LC)2	the amino acid sequence of SEQ ID NO:9;
	CDR(LC)2	the amino acid sequence of SEQ ID NO:3;
	FW(LC)3	the amino acid sequence of SEQ ID NO:10;
	CDR(LC)3	the amino acid sequence of SEQ ID NO:4;
	FW(LC)4	the amino acid sequence of SEQ ID NO:11; and
	FW(HC)1	the amino acid sequence of SEQ ID NO:12;
	CDR(HC)1	the amino acid sequence of SEQ ID NO:5;
	FW(HC)2	the amino acid sequence of SEQ ID NO:13;
	CDR(HC)2	the amino acid sequence of SEQ ID NO:6;
	FW(HC)3	the amino acid sequence of SEQ ID NO:114;
	CDR(HC)3	the amino acid sequence of SEQ ID NO:7;
	FW(HC)4	the amino acid sequence of SEQ ID NO:15;

or

(ii)	FW(LC)1	the amino acid sequence of SEQ ID NO:8;
	CDR(LC)1	the amino acid sequence of SEQ ID NO:2;
	FW(LC)2	the amino acid sequence of SEQ ID NO:9;
	CDR(LC)2	the amino acid sequence of SEQ ID NO:3;
	FW(LC)3	the amino acid sequence of SEQ ID NO:10;
	CDR(LC)3	the amino acid sequence of SEQ ID NO:4;
	FW(LC)4	the amino acid sequence of SEQ ID NO:11; and
	FW(HC)1	the amino acid sequence of SEQ ID NO:12;
	CDR(HC)1	the amino acid sequence of SEQ ID NO:5;
	FW(HC)2	the amino acid sequence of SEQ ID NO:13;
	CDR(HC)2	the amino acid sequence of SEQ ID NO:6;
	FW(HC)3	the amino acid sequence of SEQ ID NO:16;

CDR(HC)3	the amino acid sequence of SEQ ID NO:7;
FW(HC)4	the amino acid sequence of SEQ ID NO:15.

The primary structure shown in formula I represents the order of the components of the light chain variable domain of the antibody of the present invention from the N-terminus to the C-terminus. The primary structure shown in formula II represents the order of the components of the heavy chain variable domain of the antibody of the present invention from the N-terminus to the C-terminus. In each case, framework region (FW) 1 represents the most N-terminal part of the respective variable chain domain, while FW 4 represents the most C-terminal part of the respective variable chain domain.

As defined above, the respective FW and CDR sequences "comprise" the recited amino acid sequences. In one embodiment the respective FW and CDR sequences consist of said amino acid sequences, i.e. the light chain variable domain(s) and heavy chain variable domain(s) of the anti-pY_{796}-DDR1 antibody of the invention consist of the FWs and CDRs as represented in formula I and formula II, respectively, wherein the respective FW and CDR sequences consist of the recited amino acid sequences.

With regard to the CDRs and variants thereof, the above provided definitions and specifically exemplified embodiments apply *mutatis mutandis*.

With regard to the framework regions, a certain degree of variability is also envisaged herein, i.e. the individual FWs can comprise the, or consist of the specifically recited amino acid sequence or of an amino acid sequence at least 85% identical thereto. Preferably, the identity is at least 90%, more preferred at least 92.5%, more preferred at least 95%, even more preferred the identity is at least 98%, such as at least 99% and most preferably the identity is at least 99.5%. It will be appreciated that for different FWs, a different degree of sequence identity may be allowable, depending on the actual sequence and e.g. the length of the respective FW sequence, as well as its location within the respective variable chain domain.

In accordance with the present invention, the term "% sequence identity" describes the number of matches ("hits") of identical amino acids of two or more aligned amino acid sequences as compared to the number of amino acid residues making up the overall length of the amino acid sequences (or the overall compared part thereof). Percent identity is determined by dividing the

number of identical residues by the total number of residues and multiplying the product by 100. In other terms, using an alignment, the percentage of amino acid residues that are the same (e.g., 85% identity) may be determined for two or more sequences or sub-sequences when these (sub)sequences are compared and aligned for maximum correspondence over a window of comparison, or over a designated region as measured using a sequence comparison algorithm as known in the art, or when manually aligned and visually inspected.

Those having skill in the art know how to determine percent sequence identity between/among sequences using, for example, algorithms such as those based on the NCBI BLAST algorithm (Altschul, S.F. et al. [1997] Nucleic Acids Res. 25:3389-3402), CLUSTALW computer program (Tompson, J.D. et al. [1994] Nucleic Acids Res. 22:4673-4680) or FASTA (Pearson, W.R. & Lipman, D.J. [1988] Proc. Natl. Acad. Sci. U.S.A. 85:2444-2448). In one embodiment, the NCBI BLAST algorithm is employed in accordance with this invention. For amino acid sequences, the BLASTP program uses as default a word length (W) of 3, and an expectation (E) of 10. The BLOSUM62 scoring matrix (Henikoff, S. & Henikoff, J.G. [1992] Proc. Natl. Acad. Sci. U.S.A. 89:10915-10919) uses alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands. Accordingly, in those embodiments where a % sequence identity is indicated, all the amino acid sequences having a sequence identity of at least 85% as determined with the NCBI BLAST program fall under the scope of said embodiments.

The above described degree of variation in the framework regions as compared to the respective specifically recited amino acid sequence can be due to the substitution, insertion, addition, or deletion of (an) amino acid(s).

The term "substitution", has been defined herein above. In those cases where more than one amino acid is to be substituted, each amino acid is independently replaced with another amino acid, i.e. for each amino acid that is removed a different amino acid is introduced at the same position.

The term “insertion”, in accordance with the present invention, refers to the addition of one or more amino acids to the specifically recited amino acid sequence, wherein the addition is not to the N- or C-terminal end of the polypeptide.

The term “addition”, in accordance with the present invention, refers to the addition of one or more amino acids to the specifically recited amino acid sequence, either to the N- or C-terminal end of the polypeptide, or to both.

The term “deletion”, as used in accordance with the present invention, refers to the loss of one or more amino acids from the specifically recited amino acid sequence.

In one embodiment, the variation in the amino acid sequences of the framework regions is due to the substitution of (an) amino acid(s). Substitutions, as defined herein above, can be conservative amino acid substitutions or non-conservative amino acid substitutions. The definitions and specifically exemplified embodiments provided above with regard to the term "substitution" apply *mutatis mutandis*. In one embodiment, the substitutions in the framework regions are conservative amino acid substitutions.

In a further embodiment of the antibody of the invention, the antibody comprises a light chain variable domain consisting of framework regions (FW) and CDRs as represented in formula I above, and a heavy chain variable domain consisting of FWs and CDRs as represented in formula II above, wherein the FWs comprise the following amino acid sequences or a variant thereof that is at least 85% identical thereto and wherein the CDRs comprise the following amino acid sequences:

(i)
	FW(LC)1	comprises the amino acid sequence of SEQ ID NO:8;
	CDR(LC)1	comprises the amino acid sequence of SEQ ID NO:1;
	FW(LC)2	comprises the amino acid sequence of SEQ ID NO:9;
	CDR(LC)2	comprises the amino acid sequence of SEQ ID NO:3;
	FW(LC)3	comprises the amino acid sequence of SEQ ID NO:10;
	CDR(LC)3	comprises the amino acid sequence of SEQ ID NO:4;
	FW(LC)4	comprises the amino acid sequence of SEQ ID NO:11; and
	FW(HC)1	comprises the amino acid sequence of SEQ ID NO:12;
	CDR(HC)1	comprises the amino acid sequence of SEQ ID NO:5;
	FW(HC)2	comprises the amino acid sequence of SEQ ID NO:13;
	CDR(HC)2	comprises the amino acid sequence of SEQ ID NO:6;
	FW(HC)3	comprises the amino acid sequence of SEQ ID NO:14;
	CDR(HC)3	comprises the amino acid sequence of SEQ ID NO:7;
	FW(HC)4	comprises the amino acid sequence of SEQ ID NO:25;

or

(ii) FW(LC)1 comprises the amino acid sequence of SEQ ID NO:8;
CDR(LC)1 comprises the amino acid sequence of SEQ ID NO:2;
FW(LC)2 comprises the amino acid sequence of SEQ ID NO:9;
CDR(LC)2 comprises the amino acid sequence of SEQ ID NO:3;
FW(LC)3 comprises the amino acid sequence of SEQ ID NO:10;
CDR(LC)3 comprises the amino acid sequence of SEQ ID NO:4;
FW(LC)4 comprises the amino acid sequence of SEQ ID NO:11; and
FW(HC)1 comprises the amino acid sequence of SEQ ID NO:12;
CDR(HC)1 comprises the amino acid sequence of SEQ ID NO:5;
FW(HC)2 comprises the amino acid sequence of SEQ ID NO:13;
CDR(HC)2 comprises the amino acid sequence of SEQ ID NO:6;
FW(HC)3 comprises the amino acid sequence of SEQ ID NO:16;
CDR(HC)3 comprises the amino acid sequence of SEQ ID NO:7;
FW(HC)4 comprises the amino acid sequence of SEQ ID NO:15.

Where the anti-pY_{796}-DDR1 antibody of the present invention comprises one or several variations in the framework regions, the total amount of all variations present in framework regions 1 to 4 of the light chain variable domain taken together is in one embodiment at most 16 amino acid substitutions and the total amount of all variations present in framework regions 1 to 4 of the heavy chain variable domain taken together is in said embodiment at most 18 amino acid substitutions.

In a further embodiment, the CDRs consist of the above recited specific sequences (i.e. without any variations) and the above recited framework regions comprise at most the following amount of amino acid variations within the above recited specific sequences:

(i) FW(LC)1 at most 3 amino acid variations;
FW(LC)2 at most 2 amino acid variations;
FW(LC)3 at most 4 amino acid variations;
FW(LC)4 at most 1 amino acid variation; and
FW(HC)1 at most 3 amino acid variations;
FW(HC)2 at most 2 amino acid variations;
FW(HC)3 at most 4 amino acid variations; and
FW(HC)4 at most 1 amino acid variation;

or

(ii) FW(LC)1 at most 3 amino acid variations;
FW(LC)2 at most 2 amino acid variations;
FW(LC)3 at most 4 amino acid variations;
FW(LC)4 at most 1 amino acid variation; and
FW(HC)1 at most 3 amino acid variations;
FW(HC)2 at most 2 amino acid variations;
FW(HC)3 at most 4 amino acid variations; and
FW(HC)4 at most 1 amino acid variation.

In a further embodiment, the amino acid variations are substitutions.

In a further embodiment, the total amount of variations present in the light or heavy chain variable domain framework regions is at most 9 amino acid substitutions, such as e.g. at most 8 amino acid substitutions, e.g. at most 6 amino acids substitutions, such as at most 4 amino acids substitutions, e.g. at most 3 amino acids substitutions, such as at most 2 amino acids substitutions. In a further embodiment, there is only 1 amino acid substitution present in the framework regions 1 to 4 of the light chain variable domain taken together or in the framework regions 1 to 4 of the heavy chain variable domain taken together.

Because the parts of formula I and formula II defined herein as FWs are amino acid sequences that form part of the frame or scaffold of the variable chain regions, substitution within said sequences, in particular in form of conservative amino acid substitutions, will in many cases not affect the binding capability of the anti-pY_{796}-DDR1 antibody. This is because these amino acids typically are not directly involved in the binding to pY_{796}-DDR1, and their substitution for suitable alternative amino acids can be designed such that no alteration in the three-dimensional structure and folding of the protein occurs. On the other hand, such substitutions can provide numerous beneficial effects such as for improved expression in certain hosts or for stabilization of the protein by introduction of e.g. additional disulphide bridges.

The present invention further relates to an antibody comprising

(i) a light chain variable domain consisting of an amino acid sequence that is at least 85% identical to the light chain variable domain consisting of the amino acid sequence of SEQ ID NO:18, and a heavy chain variable domain consisting of an amino acid sequence that

is at least 85% identical to the heavy chain variable domain consisting of the amino acid sequence of SEQ ID NO:19; or

(ii) a light chain variable domain consisting of an amino acid sequence that is at least 85% identical to the light chain variable domain consisting of the amino acid sequence of SEQ ID NO:20, and a heavy chain variable domain consisting of an amino acid sequence that is at least 85% identical to the heavy chain variable domain consisting of the amino acid sequence of SEQ ID NO:21;

wherein the antibody specifically binds to DDR1 which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17.

All definitions and specifically exemplified embodiments provided herein above with regard to the anti-pY796-DDR1 antibody of the invention, in particular the cited degrees and types of variations apply *mutatis mutandis*.

In one embodiment in accordance with this aspect of the invention:

(i) the antibody of the invention according to option (i) binds to pY_{796}-DDR1 with an affinity that is at least the same or essentially the same as an antibody that comprises as light chain variable domain the amino acid sequence of SEQ ID NO:18 and as heavy chain variable domain the amino acid sequence of SEQ ID NO:19 (i.e. the variable chain regions designated as 20G11 in the appended examples); and/or

(ii) the antibody of the invention according to option (ii) binds to pY_{796}-DDR1 with an affinity that is at least the same or essentially the same as an antibody that comprises as light chain variable domain the amino acid sequence of SEQ ID NO:20 and as heavy chain variable domain the amino acid sequence of SEQ ID NO:21 (i.e. the variable chain regions designated as 22D9, or alternatively as 31A5, in the appended examples).

The binding affinity of the antibodies 20G11and 22D9 are provided in Table 3 herein below. Accordingly, in one embodiment in accordance with this aspect of the invention,

(i) the antibody of the invention according to option (i) binds to pY_{796}-DDR1 with a K_D of 3.8E-11 M or lower; and/or

(ii) the antibody of the invention according to option (ii) binds to pY_{796}-DDR1 with a K_D of 5.1E-11 M or lower.

The term "K_D" refers to the equilibrium dissociation constant (the reciprocal of the equilibrium binding constant) and is used herein according to the definitions provided in the art. Means and methods for determining the K_D value are as described above.

In accordance with the present invention, the binding affinity to pY_{796}-DDR1 is considered to be essentially retained if the difference or the ratio between the K_D of the antibody comprising the specifically recited light and heavy chain variable domains and the K_D of the same antibody with amino acid modifications is within two orders of magnitude, more preferably within one order of magnitude. In one embodiment, the binding affinity is fully retained, i.e. the K_D of the antibody comprising light and heavy chain variable domains with amino acid modifications is equal or lower than the K_D of the same antibody comprising the specifically recited light and heavy chain variable domains. Generally, a lower K_D value corresponds to a higher or better affinity as is well known in the art. Therefore, the invention also encompasses antibodies comprising modified amino acids that have an increased binding affinity compared to the antibody without such amino acid modifications.

In one embodiment, the anti-pY_{796}-DDR1 antibodies of the invention bind to pY_{796}-DDR1 with a K_D of 6E-11 M or lower, such as e.g. 5E-11 M or lower, or even 4E-11 M or lower, such as e.g. 3E-11 M or lower.

In a further embodiment, the antibody:

(i) comprises as light chain variable domain the amino acid sequence of SEQ ID NO:18 and as heavy chain variable domain the amino acid sequence of SEQ ID NO:19 (i.e. corresponding to the variable chain regions designated as 20G11 in the appended examples); or

(ii) comprises as light chain variable domain the amino acid sequence of SEQ ID NO:20 and as heavy chain variable domain the amino acid sequence of SEQ ID NO:21 (i.e. corresponding to the variable chain regions designated as 22D9 and as 31A5 in the appended examples).

The above recited sequences for the variable light and heavy chain regions are the amino acid sequences that have been employed in the appended examples. Full-length amino acid sequences for the light and heavy chains present in the antibodies employed in the examples (that comprise said variable domains) are represented in SEQ ID NOs: 22 to 27. The examples

provide two different antibodies comprising as light chain variable domain the amino acid sequence of SEQ ID NO:20 and as heavy chain variable domain the amino acid sequence of SEQ ID NO:21 (designated as 22D9 and as 31A5). These two antibodies differ in the Fc part of the full length sequence. As is shown in the examples, these anti-pY_{796}-DDR1 antibodies are particularly useful when used in immunohistochemical staining of cells and/or tissues for the determination of the presence or absence of a phosphorylation at tyrosine 796 of DDR1.

The present invention further relates to a nucleic acid molecule encoding a light chain variable region of any one of the antibodies of the invention defined herein above. This nucleic acid molecule is referred to herein as the first nucleic acid molecule of the invention. Furthermore, the present invention also relates to a nucleic acid molecule encoding a heavy chain variable region of any one of the antibodies of the invention defined herein above. This nucleic acid molecule is referred to herein as the second nucleic acid molecule of the invention.

In accordance with the present invention, the term "nucleic acid molecule", also referred to as nucleic acid sequence or polynucleotide herein, includes DNA, such as cDNA or genomic DNA.

The nucleic acid molecules of the invention can e.g. be synthesized by standard chemical synthesis methods and/or recombinant methods, or produced semi-synthetically, e.g. by combining chemical synthesis and recombinant methods. Ligation of the coding sequences to transcriptional regulatory elements and/or to other amino acid encoding sequences can be carried out using established methods, such as restriction digests, ligations and molecular cloning.

In accordance with the present invention, the first nucleic acid molecule of the invention encodes a light chain variable region:

(i) comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:1 or a variant thereof that differs in at most one amino acid substitution, a CDR2 comprising the amino acid sequence of SEQ ID NO:3 or a variant thereof that differs in at most one amino acid substitution, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 or a variant thereof that differs in at most one amino acid substitution;

(ii) comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2 or a variant thereof that differs in at most one amino acid substitution, a CDR2 comprising the amino

acid sequence of SEQ ID NO:3 or a variant thereof that differs in at most one amino acid substitution, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 or a variant thereof that differs in at most one amino acid substitution;

(iii) consisting of an amino acid sequence of formula I as defined herein above;

(iv) consisting of an amino acid sequence that is at least 85% identical to the light chain variable domain consisting of the amino acid sequence of SEQ ID NO:18 or;

(v) consisting of an amino acid sequence that is at least 85% identical to light chain variable domain having the amino acid sequence of SEQ ID NO:20.

Similarly, the second nucleic acid molecule of the invention encodes a heavy chain variable region:

(i) comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:5 or a variant thereof that differs in at most one amino acid substitution, a CDR2 comprising the amino acid sequence of SEQ ID NO:6 or a variant thereof that differs in at most one amino acid substitution, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7 or a variant thereof that differs in at most one amino acid substitution;

(ii) comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:5 or a variant thereof that differs in at most one amino acid substitution, a CDR2 comprising the amino acid sequence of SEQ ID NO:6 or a variant thereof that differs in at most one amino acid substitution, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7 or a variant thereof that differs in at most one amino acid substitution;

(iii) consisting of an amino acid sequence of formula II as defined herein above;

(iv) consisting of an amino acid sequence that is at least 85% identical to the heavy chain variable domain consisting of the amino acid sequence of SEQ ID NO:19; or

(vii) consisting of an amino acid sequence that is at least 85% identical to the heavy chain variable domain consisting of the amino acid sequence of SEQ ID NO:21.

The present invention further relates to a vector comprising the first nucleic acid molecule of the invention, i.e. a nucleic acid molecule encoding a light chain variable region of any one of the antibodies of the invention defined herein above. The present invention further relates to a vector comprising the second nucleic acid molecule of the invention, i.e. a nucleic acid molecule encoding a heavy chain variable region of any one of the antibodies of the invention defined herein above. Such vectors are also referred to herein as the "individual vector(s) of the invention".

Many suitable vectors are known to those skilled in molecular biology, the choice of which depends on the desired function. Non-limiting examples of vectors include plasmids, cosmids, viruses, bacteriophages and other vectors used conventionally in e.g. genetic engineering. Methods which are well known to those skilled in the art can be used to construct various plasmids and vectors; see, for example, the techniques described in Sambrook et al. (loc cit.) and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1989), (1994).

In one embodiment, the vector is an expression vector. An expression vector according to this invention is capable of directing the replication and the expression of the nucleic acid molecule of the invention in a host and, accordingly, provides for the expression of the variable chain domains of the anti-pY_{796}-DDR1 antibodies of the present invention encoded thereby in the selected host. In a further embodiment, the vector(s) comprise(s) further sequences to ensure that not only said variable chain domains of the invention are expressed, but also the full-length IgG antibodies comprising said variable chain domains of the invention.
Expression vectors can for instance be cloning vectors, binary vectors or integrating vectors. Expression comprises transcription of the nucleic acid molecule, for example into a translatable mRNA. In one embodiment, the vector is a eukaryotic expression plasmid for the transient recombinant expression of the heavy chain and/or the light chain of monoclonal rabbit antibodies. Such vectors have been specifically developed for antibody expression but also antibody production by e.g. transient transfection of eukaryotic cells e.g. HEK 293 or derivatives thereof or CHO cells.

Non-limiting examples of vectors include pQE-12, the pUC-series, pBluescript (Stratagene), the pET-series of expression vectors (Novagen) or pCRTOPO (Invitrogen), lambda gt11, pJOE, the pBBR1-MCS series, pJB861, pBSMuL, pBC2, pUCPKS, pTACT1, pTRE, pCAL-n-EK, pESP-1, pOP13CAT, the E-027 pCAG Kosak-Cherry (L45a) vector system, pREP (Invitrogen), pCEP4 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2neo, pBPV-1, pdBPVMMTneo, pRSVgpt, pRSVneo, pSV2-dhfr, pIZD35, Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pRc/CMV, pcDNA1, pcDNA3 (Invitrogen), pcDNA3.1, pSPORT1 (GIBCO BRL), pGEMHE (Promega), pLXIN, pSIR (Clontech), pIRES-EGFP (Clontech), pEAK-10 (Edge Biosystems) pTriEx-Hygro (Novagen) and pCINeo (Promega). Non-limiting examples for plasmid vectors suitable for

Pichia pastoris comprise e.g. the plasmids pAO815, pPIC9K and pPIC3.5K (all Invitrogen). Another vector suitable for expressing proteins in *Xenopus embryos*, zebrafish embryos as well as a wide variety of mammalian and avian cells is the multipurpose expression vector pCS2+.

Generally, vectors can contain one or more origins of replication (ori) and inheritance systems for cloning or expression, one or more markers for selection in the host, e.g., antibiotic resistance, and one or more expression cassettes. In addition, the coding sequences comprised in the vector can be ligated to transcriptional regulatory elements and/or to other amino acid encoding sequences using established methods. Such regulatory sequences are well known to those skilled in the art and include, without being limiting, regulatory sequences ensuring the initiation of transcription, internal ribosomal entry sites (IRES) (Owens, G.C. et al. [2001] Proc. Natl. Acad. Sci. U.S.A. 98:1471-1476) and optionally regulatory elements ensuring termination of transcription and stabilization of the transcript. Non-limiting examples for such regulatory elements ensuring the initiation of transcription comprise promoters, a translation initiation codon, enhancers, insulators and/or regulatory elements ensuring transcription termination, which are to be included downstream of the nucleic acid molecules of the invention. Further examples include Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing, nucleotide sequences encoding secretion signals or, depending on the expression system used, signal sequences capable of directing the expressed protein to a cellular compartment or to the culture medium. The vectors may also contain an additional expressible polynucleotide coding for one or more chaperones to facilitate correct protein folding.

Additional examples of suitable origins of replication include, for example, the full length ColE1, a truncated ColEI, the SV40 viral and the M13 origins of replication, while additional examples of suitable promoters include, without being limiting, the cytomegalovirus (CMV) promoter, SV40-promoter, RSV-promoter (Rous sarcome virus), the lacZ promoter, the tetracycline promoter/operator ($tet^{p/o}$), chicken β-actin promoter, CAG-promoter (a combination of chicken β-actin promoter and cytomegalovirus immediate-early enhancer), the gai10 promoter, human elongation factor 1α-promoter, AOX1 promoter, GAL1 promoter CaM-kinase promoter, the lac, trp or tac promoter, the T7 or T5 promoter, the lacUV5 promoter, the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) polyhedral promoter or a globin intron in mammalian and other animal cells. One example of

an enhancer is e.g. the SV40-enhancer. Non-limiting additional examples for regulatory elements ensuring transcription termination include the SV40-poly-A site, the tk-poly-A site, the rho-independent lpp terminator or the AcMNPV polyhedral polyadenylation signals. Further non-limiting examples of selectable markers include dhfr, which confers resistance to methotrexate (Reiss, Plant Physiol. (Life Sci. Adv.) 13 (1994), 143-149), npt, which confers resistance to the aminoglycosides neomycin, kanamycin and paromycin (Herrera-Estrella, EMBO J. 2 (1983), 987-995) and hygro, which confers resistance to hygromycin (Marsh, Gene 32 (1984), 481-485). Additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman, Proc. Natl. Acad. Sci. USA 85 (1988), 8047); mannose-6-phosphate isomerase which allows cells to utilize mannose (WO 94/20627) and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.) or deaminase from *Aspergillus terreus* which confers resistance to blasticidin S (Tamura, Biosci. Biotechnol. Biochem. 59 (1995), 2336-2338).

In a further embodiment, the vector is a eukaryotic expression plasmid containing an expression cassette consisting of a 5' CMV promoter including Intron A, and a 3` BGH polyadenylation sequence. In addition to the expression cassette, the plasmid can contain a pUC18-derived origin of replication and a beta-lactamase gene conferring ampicillin resistance for plasmid amplification in E. coli. For secretion of the antibodies, an eukaryotic leader sequence can be cloned 5' of the antibody gene.

Suitable bacterial expression hosts comprise e. g. strains derived from JM83, W3110, KS272, TG1, K12, BL21 (such as BL21(DE3), BL21(DE3)PlysS, BL21(DE3)RIL, BL21(DE3)PRARE) or Rosettaâ. For vector modification, PCR amplification and ligation techniques, see Sambrook & Russel [2001] (Cold Spring Harbor Laboratory, NY).

The nucleic acid molecules and/or vectors of the invention can be designed for introduction into cells by e.g. chemical based methods (polyethylenimine, calcium phosphate, liposomes, DEAE-dextrane, nucleofection), non chemical methods (electroporation, sonoporation, optical transfection, gene electrotransfer, hydrodynamic delivery or naturally occurring transformation upon contacting cells with the nucleic acid molecule of the invention), particle-based methods

(gene gun, magnetofection, impalefection) phage vector-based methods and viral methods. For example, expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, Semliki Forest Virus or bovine papilloma virus, may be used for delivery of the nucleic acid molecules into targeted cell population. Additionally, baculoviral systems can also be used as vector in eukaryotic expression system for the nucleic acid molecules of the invention. In one embodiment, the nucleic acid molecules and/or vectors of the invention are designed for transformation of chemical competent *E. coli* by calcium phosphate and/or for transient transfection of HEK293 and CHO by polyethylenimine- or lipofectamine-transfection.

The present invention further relates to a vector comprising:

(i) a nucleic acid molecule encoding a light chain variable domain according to option (i) defined herein above and a heavy chain variable domain according to option (i) defined herein above; or

(ii) a nucleic acid molecule encoding a light chain variable domain according to option (ii) defined herein above and a heavy chain variable domain according to option (ii) defined herein above.

In one embodiment, the vector is an expression vector.

All definitions and specifically exemplified embodiments provided herein above with regard to the vector of the invention, in particular vector types or the regulatory sequences apply *mutatis mutandis*. This second type of vector relates to a vector comprising at least two nucleic acid molecules, namely one encoding a light chain variable domain and one encoding a heavy chain variable domain. As is evident from the above combinations, the light chain variable domain and heavy chain variable domain are combined in the vector such that the expression of a functional anti-pY_{796}-DDR1 antibody of the invention is enabled. This second type of vector is also referred to herein as the "combination vector of the invention".

The present invention further relates to a host cell or non-human host comprising:

(i) the combination vector of the invention; or

(ii) the individual vector of the invention comprising the first nucleic acid molecule of the invention, i.e. a nucleic acid molecule encoding a light chain variable region in accordance with the invention and the individual vector of the invention comprising the

second nucleic acid molecule of the invention, i.e. a nucleic acid molecule encoding a heavy chain variable region of the invention, wherein these two vectors comprise the nucleic acid molecules encoding for matching light chain and heavy chain variable regions as defined in options (i) or (ii) above.

The host cell can be any prokaryotic or eukaryotic cell. The term "prokaryote" is meant to include all bacteria which can be transformed, transduced or transfected with DNA or DNA or RNA molecules for the expression of a protein of the invention. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens*, *Corynebacterium* (glutamicum), *Pseudomonas* (fluorescens), *Lactobacillus*, *Streptomyces*, *Salmonella* and *Bacillus subtilis*.

The term "eukaryotic" is meant to include yeast, higher plant, insect and mammalian cells. Typical mammalian host cells include, Hela, HEK293, H9, Per.C6 and Jurkat cells, mouse NIH3T3, NS/0, SP2/0 and C127 cells, COS cells, e.g. COS 1 or COS 7, CV1, quail QC1-3 cells, mouse L cells, mouse sarcoma cells, Bowes melanoma cells and Chinese hamster ovary (CHO) cells. Exemplary mammalian host cells in accordance with the present invention are CHO cells. Other suitable eukaryotic host cells include, without being limiting, chicken cells, such as e.g. DT40 cells, or yeasts such as *Saccharomyces cerevisiae*, *Pichia pastoris, Schizosaccharomyces pombe and Kluyveromyces lactis*. Insect cells suitable for expression are e.g. *Drosophila* S2, *Drosophila* Kc, *Spodoptera* Sf9 and Sf21 or *Trichoplusia* Hi5 cells. Suitable zebrafish cell lines include, without being limiting, ZFL, SJD or ZF4.

The described vector(s) can either integrate into the genome of the host or can be maintained extrachromosomally. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleic acid molecules, and as desired, the collection and purification of the antibody of the invention may follow. Appropriate culture media and conditions for the above described host cells are known in the art.

In one embodiment, the recited host is a mammalian cell, such as a human cell or human cell line. In a further embodiment, the host cell transformed with the vector(s) of the invention is HEK293 or CHO. In yet a further embodiment, the host cell transformed with the vector(s) of the invention is CHO. These host cells as well as suitable media and cell culture conditions have been described in the art, see e.g. Baldi L. *et al.*, Biotechnol Prog. 2005 Jan-

Feb;21(1):148-53, Girard P. *et al.*, Cytotechnology. 2002 Jan;38(1-3):15-21 and Stettler M. *et al.*, Biotechnol Prog. 2007 Nov-Dec;23(6):1340-6.

With regard to the term "vector comprising" in accordance with the present invention it is understood that further nucleic acid sequences are present in the vectors that are necessary and/or sufficient for the host cell to produce an anti-pY_{796}-DDR1 antibody of the invention. Such further nucleic acid sequences are e.g. nucleic acid sequences encoding the remainder of the light chain as well as nucleic acid sequences encoding the remainder of the heavy chain.

The host cell or non-human host, in accordance with the present invention, comprises either one vector encoding both the light chain and heavy chain variable regions as defined herein above or it comprises two separate vectors, wherein one vector carries a nucleic acid molecule encoding a light chain variable region in accordance with the present invention and the second vector carries a nucleic acid molecule encoding a matching heavy chain variable region in accordance with the present invention. Thus, where the first vector carries a nucleic acid molecule encoding a light chain variable region in accordance with option (i) herein above, then the second vector carries a nucleic acid molecule encoding a heavy chain variable region also in accordance with option (i) above. The same applies *mutatis mutandis* to option (ii).

Accordingly, in each case, expression of those nucleic acid molecules is linked to each other that are required to be present within one antibody molecule to ensure the production of a anti-pY_{796}-DDR1 antibody of the invention having the binding capabilities described herein above.

The host cells in accordance with this embodiment may e.g. be employed to produce large amounts of the anti-pY_{796}-DDR1 antibodies of the present invention. Said host cells are produced by introducing the above described vector(s) into the host. The presence of said vector(s) in the host then mediates the expression of the nucleic acid molecules encoding the above described light chain variable domains and heavy chain variable domains of the anti-pY_{796}-DDR1 antibodies of the invention. As described herein above, the vector(s) of the invention can comprise further sequences enabling the expression of full length IgG antibodies, thereby resulting in the production of full length IgG antibodies by the host cells, wherein said antibodies are characterized by the presence of the variable light and/or heavy chain domains in accordance with the present invention.

The present invention further relates to a method for the production of an antibody that specifically binds to DDR1 which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17, the method comprising culturing the host cell of the invention under suitable conditions and isolating the antibody produced.

In accordance with this embodiment, the vector(s) present in the host of the invention is/are either (an) expression vector(s), or the vector(s) mediate(s) the stable integration of the nucleic acid molecule(s) of present invention into the genome of the host cell in such a manner that expression thereof is ensured. Means and methods for selection a host cell in which the nucleic acid molecules encoding the respective light and heavy chain domains of the anti-pY_{796}-DDR1 antibody of the present invention have been successfully introduced such that expression of the antibody is ensured are well known in the art and have been described (Browne, S.M. & Al-Rubeai, M. [2007] Trends Biotechnol. 25:425-432; Matasci, M et al. [2008] Drug Discov. Today: Technol. 5:e37-e42; Wurm, F.M. [2004] Nat. Biotechnol. 22:1393-1398).

Suitable conditions for culturing prokaryotic or eukaryotic host cells are well known to the person skilled in the art. For example, bacteria such as e.g. *E. coli* can be cultured under aeration in Luria Bertani (LB) medium, typically at a temperature from 4 to about 37°C. To increase the yield and the solubility of the expression product, the medium can be buffered or supplemented with suitable additives known to enhance or facilitate both. In those cases where inducible promoters control the nucleic acid molecules of the invention in the vector(s) present in the host cell, expression of the polypeptide can be induced by addition of an appropriate inducing agent, such as e.g. anhydrotetracycline. Suitable expression protocols and strategies have been described in the art (e.g. in Dyson, M. R., *et al.* (2004). BMC Biotechnol. 4, 32–49 and in Baldi, L. *et al.* (2007). Biotechnol. Lett. 29, 677–684) and can be adapted to the needs of the specific host cells and the requirements of the protein to be expressed, if required.

Depending on the cell type and its specific requirements, mammalian cell culture can e.g. be carried out in RPMI, Williams' E or DMEM medium containing 10% (v/v) FCS, 2 mM L-glutamine and 100 U/ml penicillin/streptomycin. The cells can be kept e.g. at 37°C or at 41°C for DT40 chicken cells, in a 5% CO_2, water-saturated atmosphere.
A suitable medium for insect cell culture is e.g. TNM + 10% FCS, SF900 or HyClone SFX-Insect medium. Insect cells are usually grown at 27°C as adhesion or suspension cultures.

Suitable expression protocols for eukaryotic or vertebrate cells are well known to the skilled person and can be retrieved e.g. from Sambrook, J & Russel, D.W. [2001] (Cold Spring Harbor Laboratory, NY).

In one embodiment, the method is carried out using mammalian cells, such as e.g. CHO or HEK293 cells. In a further embodiment, the method is carried out using CHO cells.

Depending upon the host employed in a recombinant production procedure, the antibody expressed may be glycosylated or may be non-glycosylated. In one embodiment, a plasmid or a virus is used containing the coding sequence of the antibody of the invention and genetically fused thereto an N-terminal FLAG-tag and/or C-terminal His-tag. In a further embodiment, the length of said FLAG-tag is about 4 to 8 amino acids, such as e.g. exactly 8 amino acids. An above described vector can be used to transform or transfect the host using any of the techniques commonly known to those of ordinary skill in the art. Furthermore, methods for preparing fused, operably linked genes and expressing them in, e.g., mammalian cells and bacteria are well-known in the art (Sambrook, loc cit.).

The transformed hosts can be grown in bioreactors and cultured according to techniques known in the art to achieve optimal cell growth. The antibody of the invention can then be isolated from the growth medium. The isolation and purification of the, e.g., microbially expressed antibodies of the invention may be by any conventional means such as, e.g., affinity chromatography (for example using a fusion-tag such as the *Strep*-tag II or the His_6 tag), gel filtration (size exclusion chromatography), anion exchange chromatography, cation exchange chromatography, hydrophobic interaction chromatography, high pressure liquid chromatography (HPLC), reversed phase HPLC or immunoprecipitation. These methods are well known in the art and have been generally described, e.g. in Sambrook, J & Russel, D.W. [2001] (Cold Spring Harbor Laboratory, NY).

It will be appreciated that in accordance with the present invention, the term "isolating the antibody produced" refers to the isolation of the anti-pY_{796}-DDR1 antibody of the present invention.

Furthermore, the present invention relates to an antibody that specifically binds to DDR1 which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in

SEQ ID NO:17, wherein the antibody is obtainable or obtained by the method of the invention.

The present invention further relates to a composition comprising at least one of:

(i) the antibody of the invention,

(ii) the nucleic acid molecule of the invention,

(iii) the vector of the invention,

(iv) the host cell of the invention, and/or

(v) the antibody produced by the method of the invention.

The term "composition", as used in accordance with the present invention, relates to a composition which comprises at least one of the recited compounds. It may, optionally, comprise further molecules capable of altering the characteristics of the compounds of the invention thereby, for example, stabilizing, modulating and/or enhancing their function. The composition may be in solid or liquid form and may be, inter alia, in the form of (a) powder(s), (a) tablet(s) or (a) solution(s).

The components of the composition can be packaged in a container or a plurality of containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of 1% (w/v) or 10% (w/v) aqueous solution, and the resulting mixture is lyophilized. A solution for use is prepared by reconstituting the lyophilized compound(s) using either e.g. water-for-injection for therapeutic uses or another desired solvent, e.g. a buffer, for diagnostic purposes. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.
The various components of the composition may be packaged as a kit with instructions for use.

In one embodiment, the composition of the invention is a composition enabling the skilled person to carry out *in vivo* as well as in *in vitro* or *ex vivo* methods well known in the art. For example, methods such as e.g. immunohistochemical staining of tissues or cells obtained from a patient or measuring the amount of anti-pY_{796}-DDR1 antibody in a particular tissue can be of value. The anti-pY_{796}-DDR1 antibodies provided herein are also suitable for use in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. Examples of immunoassays which can utilize the antibodies of the invention are immunoassays in either a direct or indirect format. Examples of such immunoassays are the

enzyme linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), the Western blot assay, or immuno assays based on detection of luminescence, fluorescence, chemiluminescence or electrochemiluminescence.

The present invention further relates to the use of the antibody of the invention for determining phosphorylation of DDR1 at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17.

As described herein above, the anti-pY_{796}-DDR1 antibody of the present invention specifically binds to DDR1 when it is phosphorylated at the tyrosine in position 796, but does not or essentially does not cross-react with a different protein, in particular a different protein of similar structure, or with DDR1 that is not phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17. Accordingly, said antibody is suitable for determining the phosphorylation of DDR1 at said tyrosine at position 796.

The skilled person is well aware of how to determine whether a particular protein, here DDR1, is phosphorylated by using an antibody specific for said phosphorylation status. Non-limiting examples of suitable methods of determining the phosphorylation of DDR1 at said tyrosine at position 796 by employing the anti-pY_{796}-DDR1 antibody of the invention include, without being limiting, immunohistochemical and immunocytochemical methods, Western blotting, ELISA, and immuno assays based on detection of luminescence, fluorescence, chemiluminescence or electrochemiluminescence. In one embodiment, the determination of phosphorylation of DDR1 is by immunohistochemistry.

Furthermore, the present invention relates to a method of determining phosphorylation of DDR1 at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17, the method comprising detecting the binding of the antibody of the invention to DDR1.

Due to the suitability of the anti-pY_{796}-DDR1 antibody of the invention for determining the phosphorylation of DDR1 at the tyrosine in position 796, it can be employed in a method of determining said phosphorylation, as discussed above. To this end, a sample containing DDR1 is brought in contact with the antibody of the invention and the binding of the anti-pY_{796}-DDR1 antibody to DDR1 is detected. If the antibody binds to DDR1, then said DDR1 is phosphorylated. If no binding occurs, then the DDR1 is not phosphorylated at the tyrosine in

position 796. In one embodiment, the method includes the use of suitable controls to ensure that any positive (i.e. phosphorylation is detected) or negative result (i.e. no phosphorylation is detected) represents a correct result. Suitable positive as well as negative controls can be designed and included in the experimental setup by a skilled person without further ado and include e.g. DDR1 preparations known to have a phosphorylation at tyrosine 796, for example due to the application of a previous phosphorylation step, as positive control, as well as DDR1 preparations known to not have a phosphorylation at tyrosine 796, for example due to the application of a previous de-phosphorylation step, as negative control.

These and other embodiments are disclosed and encompassed by the description and Examples of the present invention. Further literature concerning any one of the methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries and databases, using for example electronic devices. For example, the public database "Medline", available on the Internet, may be utilized, for example in the World Wide Web under ncbi.nlm.nih.gov/PubMed/medline.html. Further databases and addresses available in the World Wide Web, such as ncbi.nlm.nih.gov/, fmi.ch/biology/research_tools.html,tigr.org/, or infobiogen.fr/, are known to the person skilled in the art and can also be obtained using the address in the World Wide Web under lycos.com.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the patent specification, including definitions, will prevail.

All amino acid sequences provided herein are presented starting with the most N-terminal residue and ending with the most C-terminal residue (N→C), as customarily done in the art, and the one-letter or three-letter code abbreviations as used to identify amino acids throughout the present invention correspond to those commonly used for amino acids.

Regarding the embodiments characterized in this specification, in particular in the claims, it is intended that each embodiment mentioned in a dependent claim is combined with each embodiment of each claim (independent or dependent) said dependent claim depends from. For example, in case of an independent claim 1 reciting 3 alternatives A, B and C, a dependent claim 2 reciting 3 alternatives D, E and F and a claim 3 depending from claims 1 and 2 and reciting 3 alternatives G, H and I, it is to be understood that the specification unambiguously

discloses embodiments corresponding to combinations A, D, G; A, D, H; A, D, I; A, E, G; A, E, H; A, E, I; A, F, G; A, F, H; A, F, I; B, D, G; B, D, H; B, D, I; B, E, G; B, E, H; B, E, I; B, F, G; B, F, H; B, F, I; C, D, G; C, D, H; C, D, I; C, E, G; C, E, H; C, E, I; C, F, G; C, F, H; C, F, I, unless specifically mentioned otherwise.

Similarly, and also in those cases where independent and/or dependent claims do not recite alternatives, it is understood that if dependent claims refer back to a plurality of preceding claims, any combination of subject-matter covered thereby is considered to be explicitly disclosed. For example, in case of an independent claim 1, a dependent claim 2 referring back to claim 1, and a dependent claim 3 referring back to both claims 2 and 1, it follows that the combination of the subject-matter of claims 3 and 1 is clearly and unambiguously disclosed as is the combination of the subject-matter of claims 3, 2 and 1. In case a further dependent claim 4 is present which refers to any one of claims 1 to 3, it follows that the combination of the subject-matter of claims 4 and 1, of claims 4, 2 and 1, of claims 4, 3 and 1, as well as of claims 4, 3, 2 and 1 is clearly and unambiguously disclosed.

The above considerations apply mutatis mutandis to all appended claims. To give a non-limiting example, the combination of claims 10, 9 and 1(i) is clearly and unambiguously envisaged in view of the claim structure. The same applies for example to the combination of claims 10, 9 and 4(ii), etc..

The invention is illustrated with the following figures which show:

Figure 1: Detection of pDDR1 in IHC FFPE control cell lines. Phosphorylation was induced by stimulation of the U2OS cells with collagen. Subsequent to stimulation the cells were fixed and paraffin embedded. It is shown that the selected clones can detect phosphorylated DDR1 (lower panel), whereas no signal is obvious in the unstimulated control cells (upper panel). Membrane staining of phosphorylated DDR1 is highlighted with black arrows (lower panel).

Figure 2: Detection of DDR1 and pDDR1, respectively, by Western Blotting. U2OS cells were treated with collagen to stimulate the cells and to induce DDR1 phosphorylation. For the negative control lysates, untreated U2OS cells were used. Subsequent to stimulation the cells were lyzed, subjected to SDS-PAGE, transferred to nitrocellulose and analyzed for presence of DDR1 and pDDR1, respectively by probing with the antibodies depicted in the Figure.

The following Examples illustrate the invention:

Example 1: Materials & general methods

Recombinant DNA techniques

Standard methods were used to manipulate DNA as described in Sambrook, J. et al., Molecular Cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. The molecular biological reagents were used according to the manufacturer's instructions.

DNA sequence determination

DNA sequences were determined by double strand sequencing performed at Microsynth AG (Balgach, Switzerland).

DNA and protein sequence analysis and sequence data management

Vector NT1 Advance suite version 11.5.0 was used for sequence creation, mapping, analysis, annotation and illustration.

Example 2: Synthesis of peptide immunogens and screening reagents

Peptides were synthesized by means of fluorenylmethyloxycarbonyl (Fmoc) solid phase peptide synthesis on a multiple peptide synthesizer e.g. from Protein Technologies, Inc. For this, 4.0 equivalents of each amino acid derivative were used. Amino acid derivatives were dissolved in dimethylformamide containg 1 equivalent of 1-Hydroxy-7-azabenzotriazol. Peptides were synthesized on Tentagel R resin. Coupling reactions were carried out for 5 minutes in dimethylformamide as a reaction medium with 4 equivalents HATU and 8 equivalents of N,N-Diisopropylethylamine relative to resin loading. The Fmoc group was cleaved in 8 minutes after each synthesis step using 25% piperidine in dimethyl formamide. Release of the peptide from the synthesis resin and the cleavage of the acid-labile protecting groups was achieved in 3 hours at room temperature with 9,5 ml trifluoroacetic acid, 0.25 ml triisopropylsilane, and 0,25 ml water. The reaction solution was subsequently mixed with cooled diisopropyl ether to precipitate the peptide. The precipitate was filtered, washed again with diisopropyl ether, dissolved in a small amount of aqueous acetic acid and lyophilized. The crude material obtained was purified by preparative RP-HPLC using a gradient of

acetonitrile/water containing 0.1% trifluoroacetic acid. The identity of the purified material was checked by means of ion spray mass spectrometry. Cysteine-containing peptides were conjugated to maleimide-activated keyhole limpet hemocyanin.

The following peptides were synthesized:

Immunogen DDR1_HUMAN(788-808)[pY796]

Sequence: *Ac-*SRNLYAGD*pY*YRVQGRAVLPIR*ßAla*Ahx*ßAla*C*-NH2* (SEQ ID NO:28)

Screening Peptide DDR1_HUMAN(788-808)[pY796]

Sequence: *H-**Glu(Bi-PEG)*SRNLYAGD*pY*YRVQGRAVLPIR*-NH2* (SEQ ID NO:29)

Screening Peptide DDR1_HUMAN(788-808)

Sequence: *H-**Glu(Bi-PEG)* SRNLYAGDYYRVQGRAVLPIR*-NH2* (SEQ ID NO:30)

Example 3: Immunization of rabbits for generation of anti-phospho-DDR1 antibodies

For the generation of antibodies against the pY796-DDR1, 16-week old ZiKa rabbits were immunized with the peptide DDR1_HUMAN(788-808) [pY796] coupled to KLH. All rabbits were subjected to repeated immunizations. In the first month the animals were immunized weekly. From the second month onward the animals were immunized once per month. For each immunization 500 µg KLH-coupled peptide was dissolved in 1 mL 140 mM NaCl and was emulsified in 1 ml CFA. The development of titers was evaluated on days 45 and 105 after start of the immunization. When titers against the immunogen were detectable by ELISA, antibodies were obtained by B-cell cloning as described in Seeber et al. (2014), PLoS One. 2014 Feb 4;9(2). For the production of full-length rabbit IgG the heavy and light chain coding plasmids, derived from the recombinant IgG cloning process were used for transient transfection of HEK293 cells.

Example 4: Titer analysis

For the determination of the serum titers and to evaluate whether the polyclonal titers can discriminate between the phosphorylated and the non-phosphorylated form of the respective immunogen, a biotinylated variant of the phosphorylated screening peptide

DDR1_HUMAN(788-808) [pY796] or of the non-phosphorylated screening peptide DDR1_HUMAN(788-808) was coupled to Streptavidin coated 96-well plates. A small amount of serum of each rabbit was collected on day 45 and day 105 after the start of the immunization campaign. The biotinylated peptides were immobilized on plate surfaces at a concentration of 15 ng/mL. The sera from each rabbit were diluted in PBS with 1% BSA and the dilutions were added to the plates. The sera were tested at dilutions 1:300, 1:900, 1:2700, 1:8100, 1:24300, 1:72900, 1:218700 and 1:656100. Bound antibody was detected with a HRP-labeled $F(ab`)_2$ goat anti-rabbit Fcγ (Dianova) and ABTS (Roche) as a substrate. The titer of the analyzed animals was set by 50% signal decrease of the dilution curve. The serum titers found against the different antigens analyzed, indicate that the polyclonal antibodies from these immunized animals can distinguish between the phosphorylated and the non-phosphorylated form of the screening peptides. The titers determined are summarized in Table 1 below.

animal	DDR1_HUMAN(788-808)[pY796]	DDR1_HUMAN(788-808)
K6017	278488	19508
K6018	140873	14168
K6019	180008	7707

Table 1: The titers against the different screening reagent are shown. The titer levels indicate that the sera show specificity for the phosphorylated forms of the screening reagents.

Example 5: Development of pY796-DDR1 monoclonal antibodies

The antibodies against the peptide immunogens DDR1_HUMAN(788-808) [pY796] were obtained using the B-cell PCR method, as described in Seeber et al. (2014), PLoS One. 2014 Feb 4;9(2). PBMCs and B-cells for the single cell deposition were prepared from the rabbits at different time points. The individual pY796-DDR1 rabbit antibodies were recombinantly expressed in HEK293 cells. For the production of full-length rabbit anti pY796-DDR1 IgG the heavy and light chain coding plasmids, derived from the recombinant IgG cloning process were used for transient transfection of HEK293 cells. HEK293 cells were grown in a shaking device at 125 rpm in F17-medium (Gibco) at 37°C in an atmosphere containing 8% CO_2. Cells were split the day before transfection and seeded at a density of 0.7 - $0.8x10^6$ cells/ml. On the day of transfection, 1 - $1.5x10^6$ HEK293 cells in a volume of 2 ml were transfected with 0.5 mg heavy chain plasmid plus 0.5 mg light chain plasmid, suspended in 80 ml OptiMEMH medium

(Gibco) and supplemented with 1 ml PEIpro transfection reagent (Polyplus-Transfection) in 48-well deep well plates. Cultures were incubated for 7 days at 180 rpm at 37 °C and in 8% CO_2. After 7 days the culture supernatants were harvested and analyzed for antibody content and specificity. To test for specificity, the antibodies were tested in an ELISA format. To this end, the biotinylated variant of the phosphorylated screening peptide DDR1_HUMAN(788-808) [pY796] or of the non-phosphorylated screening peptide DDR1_HUMAN(788-808) were coupled to Streptavidin coated 96-well plates. 30 µl of the transfection supernatant of each antibody was added to the plate and incubated for 30 min at room temperature. The biotinylated peptides were immobilized on plate surfaces at a concentration of 15 ng/mL. After washing, bound antibody was detected with a HRP-labeled $F(ab`)_2$ goat anti-rabbit Fcγ (Dianova) and ABTS (Roche) as a substrate. The results are summarized in Table 2 below.

clone	DDR1_HUMAN(788-808)[pY796]	DDR1_HUMAN(788-808)
20G11	3.058	0.313
22D9	3.124	0.286
31A5	2.981	0.253

Table 2: Detection of the different screening peptides by the developed antibodies. The OD values (OD 405) are shown. From the results it is evident that the clones are specific for the phosphorylated forms of the screening peptides.

Example 6: Immunohistochemical (IHC) analysis of pY796-DDR1 antibodies

Selected clones were further tested for reactivity and specificity in IHC. To this end, 5 x10^6 cells were stimulated with collagen to achieve DDR1 phosphorylation. After the collagen treatment, the stimulated and the non-stimulated cells, respectively, were harvested, fixed in formalin and embedded in Agarose for the generation of IHC controls. After an additional fixation in formalin overnight the Agarose blocks were embedded in paraffin. After paraffin embedding, 3 µm thin sections were prepared using a microtome. The sections were mounted on glass microscopy slides and dried for 2 h. All further steps of the immunohistochemical staining procedure were carried out using a Ventana Benchmark XT (Roche). The slides were dewaxed and antigen retrieval was performed by applying heat for 1 hour. For antigen retrieval the Ventana buffer CC1 was used. Appropriate concentrations of antibodies were manually applied on the slides and incubated for 32min at 37°C. The linker and the secondary antibody

were applied by the device and incubated for 8min at RT each. Slides were counterstained with hematoxillin II and bluing reagent for 8min. After the staining procedure silicone oil was removed by washing the slides with water containing detergent. Slides were dehydrated in an ascending ethanol row and xylene and mounted with Eukitt. The results are depicted in Figure 1, which shows a specific staining of phosphorylated DDR1 with the antibodies of the invention, whereas no signal is obtained in the unstimulated control cells.

Example 7: Kinetic screening/ binding properties of pY796-DDR1 antibodies

A Biacore T200 instrument (GE Healthcare) was used to kinetically assess the binding behavior of rabbit monoclonal antibodies towards the respective phosphorylated peptide analyte:

Screening Peptide DDR1_HUMAN(788-808)[pY796]

Sequence: *H-**Glu(Bi-PEG)*SRNLYAGD*pY*YRVQGRAVLPIR*-NH2* (SEQ ID NO:31)

Rabbit IgG (150 kDa) monoclonal antibodies 20G11, 22D9, 31A5 and Rb-N-IgG (rabbit normal IgG, Sigma) were investigated for their binding specificity and affinity. A CM5 series sensor was mounted into the system and was normalized in HBS-ET buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% w/v Tween 20) according to the manufacturer's instructions. The sample buffer was the system buffer supplemented with 1 mg/ml Carboxymethyldextran (CMD, Sigma #86524). The system was operated at 25 °C.

12000 RU goat anti rabbit Fcγ (GARbFcγ), Code Nr.: 111-005-046, Jackson Immuno Research were immobilized according to the manufacturer's instructions using EDC/NHS chemistry on all four flow cells. The sensor was deactivated using 1M ethanolamine. The binding activities of the respective antibodies against the analytes were kinetically tested. Antibodies were captured at 125 nM concentration by a 30 sec injection at 10 µl/min. The flow rate was set to 100 µl/min. Analytes for which the affinity was to be tested were injected for 2 min in solution at different concentration steps of 0 nM buffer control, 0.1 nM, 0.3 nM, 1 nM, 3 nM twice, 9 nM and 27 nM. The dissociation was monitored for 9 min 30 sec.

Analytes that were employed to control the specificity of the antibody interactions were injected for 2 min at higher concentrations starting at 0 nM buffer control, 0.5 nM, 1.5 nM, 5 nM twice, 14 nM, 42 nM, 125 nM. The dissociation was monitored for 5 min. After each

analyte injection, the antibody capture system was fully regenerated by a 15 sec injection of HBS-ET buffer at 20 µl/min, followed by a 15 sec injection at 20 µl/min with 10 mM glycine buffer pH 1.5 and two injections for 1 min at 20 µl/min with 10 mM glycin pH 1.7. Were possible, kinetic signatures were evaluated according to a Langmuir fit with RMAX local.

The results are shown in Table 3 below:

mAb	CL RU	analytes	k_a 1/Ms	SE_{ka} ± 1/Ms	k_d 1/s	SE_{kd} ± 1/s	K_D M	SD_{KD} ± M	R_{max} RU	MR	Chi^2 RU^2
20G11	1087	pY796(788-808)	4.1E+06	7.5E+03	1.6E-04	3.3E-07	3.8E-11	1.1E-13	49	2.2	0.1
22D9	1218		4.6E+06	2.0E+04	2.2E-04	6.8E-07	4.8E-11	2.6E-13	50	2.0	0.1
31A5	1279		3.7E+06	8.4E+03	1.9E-04	4.3E-07	5.1E-11	1.6E-13	56	2.1	0.11
Rb-N-IgG	878		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3	0.2	0.0
buffer	5		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1	0.0	0.0

Table 3: Results of kinetic screening (n=3). mAb: antibody identity code, CL antibody capture level [RU], ka association rate constant [M-1s-1], kd dissociation rate constant [s-1], KD equilibrium dissociation constant [M], Rmax maximum analyte binding capacity [RU], MR Molar Ratio MR = (Antigen Binding RU)/Antibody Capture Level (RU)) × (MW(antibody)/ MW (antigen)). The Chi2-test shows information about the quality of the fitting model. For the interpretation of Chi2 data of real time SPR experiments refer to Onell, A. and Andersson, K.; Kinetic determinations of molecular interactions using Biacore - minimum data requirements for efficient experimental design. J.Mol.Recognit. (18): 307-317; 2005.

The Molar Ratio (MR) values indicate a fully functional 1:2 antibody to analyte binding ratio. All antibodies show subnanomolar high affinities K_D with fast association rates k_a and slow dissociation rates k_d at 25 °C.

Example 8: Western blot analysis of pY796-DDR1 antibodies

For the SDS-PAGE, U2OS cells were treated with collagen to stimulate the cells and to induce DDR1 phosphorylation. For the negative control lysates, untreated U2OS cells were used. Subsequently to the stimulation, the cells were lysed using 1x Cell Lysis buffer (Cell Signaling). Prior to the SDS-PAGE the cell lysates were incubated at 95 °C for 10 min, centrifuged and 20µg total protein was loaded on 4-12 % precast NuPAGE Bis-Tris Mini polyacrylamide gels (Invitrogen). For the protein separation, the gels were run for 15 min at 100 V and 60 min at 150 V in MOPS- running buffer (Invitrogen), using the XCell SureLock

Elektrophoresis Cell (Invitrogen Novex Mini Cell System). Proteins were transferred to nitrocellulose-membranes for 8 min at 20V using the iBlot System (Invitrogen Novex). For the detection of phosphorylated or non-phosphorylated DDR1, the membranes were incubated in blocking buffer (1 x TBST, 5 % BSA) for 1h at RT. After removing the blocking buffer, membranes were incubated for 1h at RT in 1 x TBST, 2.5 % BSA supplemented with an appropriate amount of primary antibody. Membranes were washed six times for 10 min in 1 x TBST and incubated for 1 h at room temperature in 1 x TBST, 2.5 % BSA containing secondary antibody (1:1000). The membranes were washed three times for 10 min with 1 x TBST. For the detection a horse radish peroxidase (HRP) coupled secondary antibody was used and detection was done with Luminol Western blotting detection kit (Roche) by 5 min incubation at RT.

The results are depicted in Figure 2, which shows that the antibodies of the present invention (clones 20G11, 22D9 and 31A5) specifically recognize DDR1 in its phosphorylated form, whereas the commercially available anti-total DDR1 antibody D1G6 (Cell Signaling) recognizes both the phosphorylated as well as the non-phosphorylated form of DDR1.

Claims

1. An antibody that specifically binds to the discoidin domain receptor 1 (DDR1) which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17, wherein the antibody is characterized in that the CDRs comprise the following amino acid sequences or a variant thereof that differs in at most one amino acid substitution

   (i) in the light chain variable domain a CDR1 comprising the amino acid sequence of SEQ ID NO:1, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4, and
   in the heavy chain variable domain a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7; or

   (ii) in the light chain variable domain a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4, and
   in the heavy chain variable domain a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7.

2. The antibody according to claim 1, wherein the antibody comprises a light chain variable domain consisting of framework regions (FW) and CDRs as represented in formula I:

   FW(LC)1 – CDR(LC)1 – FW(LC)2 – CDR(LC)2 – FW(LC)3 – CDR(LC)3 – FW(LC)4 (formula I)

   and a heavy chain variable domain consisting of FWs and CDRs as represented in formula II:

   FW(HC)1 – CDR(HC)1 – FW(HC)2 – CDR(HC)2 – FW(HC)3 – CDR(HC)3 –

FW(HC)4 (formula II),

wherein the FWs comprise the following amino acid sequences or a variant thereof that is at least 85% identical thereto and wherein the CDRs comprise the following amino acid sequences or a variant thereof that differs in at most one amino acid substitution:

(i) FW(LC)1 the amino acid sequence of SEQ ID NO:8;
CDR(LC)1 the amino acid sequence of SEQ ID NO:1;
FW(LC)2 the amino acid sequence of SEQ ID NO:9;
CDR(LC)2 the amino acid sequence of SEQ ID NO:3;
FW(LC)3 the amino acid sequence of SEQ ID NO:10;
CDR(LC)3 the amino acid sequence of SEQ ID NO:4;
FW(LC)4 the amino acid sequence of SEQ ID NO:11; and
FW(HC)1 the amino acid sequence of SEQ ID NO:12;
CDR(HC)1 the amino acid sequence of SEQ ID NO:5;
FW(HC)2 the amino acid sequence of SEQ ID NO:13;
CDR(HC)2 the amino acid sequence of SEQ ID NO:6;
FW(HC)3 the amino acid sequence of SEQ ID NO:14;
CDR(HC)3 the amino acid sequence of SEQ ID NO:7;
FW(HC)4 the amino acid sequence of SEQ ID NO:15;

or

(ii) FW(LC)1 the amino acid sequence of SEQ ID NO:8;
CDR(LC)1 the amino acid sequence of SEQ ID NO:2;
FW(LC)2 the amino acid sequence of SEQ ID NO:9;
CDR(LC)2 the amino acid sequence of SEQ ID NO:3;
FW(LC)3 the amino acid sequence of SEQ ID NO:10;
CDR(LC)3 the amino acid sequence of SEQ ID NO:4;
FW(LC)4 the amino acid sequence of SEQ ID NO:11; and
FW(HC)1 the amino acid sequence of SEQ ID NO:12;
CDR(HC)1 the amino acid sequence of SEQ ID NO:5;
FW(HC)2 the amino acid sequence of SEQ ID NO:13;
CDR(HC)2 the amino acid sequence of SEQ ID NO:6;
FW(HC)3 the amino acid sequence of SEQ ID NO:16;
CDR(HC)3 the amino acid sequence of SEQ ID NO:7;
FW(HC)4 the amino acid sequence of SEQ ID NO:15.

3. The antibody according to claim 1 or 2, wherein the antibody comprises a light chain variable domain consisting of framework regions (FW) and CDRs as represented in formula I:

FW(LC)1 – CDR(LC)1 – FW(LC)2 – CDR(LC)2 – FW(LC)3 – CDR(LC)3 – FW(LC)4 (formula I)

and a heavy chain variable domain consisting of FWs and CDRs as represented in formula II:

FW(HC)1 – CDR(HC)1 – FW(HC)2 – CDR(HC)2 – FW(HC)3 – CDR(HC)3 – FW(HC)4 (formula II),

wherein the FWs comprise the following amino acid sequences or a variant thereof that is at least 85% identical thereto and wherein the CDRs comprise the following amino acid sequences:

(i) FW(LC)1 comprises the amino acid sequence of SEQ ID NO:8;
CDR(LC)1 comprises the amino acid sequence of SEQ ID NO:1;
FW(LC)2 comprises the amino acid sequence of SEQ ID NO:9;
CDR(LC)2 comprises the amino acid sequence of SEQ ID NO:3;
FW(LC)3 comprises the amino acid sequence of SEQ ID NO:10;
CDR(LC)3 comprises the amino acid sequence of SEQ ID NO:4;
FW(LC)4 comprises the amino acid sequence of SEQ ID NO:11; and
FW(HC)1 comprises the amino acid sequence of SEQ ID NO:12;
CDR(HC)1 comprises the amino acid sequence of SEQ ID NO:5;
FW(HC)2 comprises the amino acid sequence of SEQ ID NO:13;
CDR(HC)2 comprises the amino acid sequence of SEQ ID NO:6;
FW(HC)3 comprises the amino acid sequence of SEQ ID NO:14;
CDR(HC)3 comprises the amino acid sequence of SEQ ID NO:7;
FW(HC)4 comprises the amino acid sequence of SEQ ID NO:15;

or

(ii) FW(LC)1 comprises the amino acid sequence of SEQ ID NO:8;
CDR(LC)1 comprises the amino acid sequence of SEQ ID NO:2;

FW(LC)2 comprises the amino acid sequence of SEQ ID NO:9;
CDR(LC)2 comprises the amino acid sequence of SEQ ID NO:3;
FW(LC)3 comprises the amino acid sequence of SEQ ID NO:10;
CDR(LC)3 comprises the amino acid sequence of SEQ ID NO:4;
FW(LC)4 comprises the amino acid sequence of SEQ ID NO:11; and
FW(HC)1 comprises the amino acid sequence of SEQ ID NO:12;
CDR(HC)1 comprises the amino acid sequence of SEQ ID NO:5;
FW(HC)2 comprises the amino acid sequence of SEQ ID NO:13;
CDR(HC)2 comprises the amino acid sequence of SEQ ID NO:6;
FW(HC)3 comprises the amino acid sequence of SEQ ID NO:16;
CDR(HC)3 comprises the amino acid sequence of SEQ ID NO:7;
FW(HC)4 comprises the amino acid sequence of SEQ ID NO:15.

4. An antibody comprising

(i) a light chain variable domain consisting of an amino acid sequence that is at least 85% identical to the light chain variable domain consisting of the amino acid sequence of SEQ ID NO:18, and

a heavy chain variable domain consisting of an amino acid sequence that is at least 85% identical to the heavy chain variable domain consisting of the amino acid sequence of SEQ ID NO:19; or

(ii) a light chain variable domain consisting of an amino acid sequence that is at least 85% identical to the light chain variable domain consisting of the amino acid sequence of SEQ ID NO:20, and

a heavy chain variable domain consisting of an amino acid sequence that is at least 85% identical to the heavy chain variable domain consisting of the amino acid sequence of SEQ ID NO:21;

wherein the antibody specifically binds to DDR1 which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17.

5. A nucleic acid molecule encoding a light chain variable region of any one of claims 1 to 4.

6. A nucleic acid molecule encoding a heavy chain variable region of any one of claims 1 to 4.

7. A vector comprising the nucleic acid molecule of claim 5.

8. A vector comprising the nucleic acid molecule of claim 6.

9. A vector comprising:
   (i) a nucleic acid molecule encoding a light chain variable region of any one of claims 1(i) to 4(i) and a heavy chain variable region of any one of claims 1(i) to 4(i); or
   (ii) a nucleic acid molecule encoding a light chain variable region of any one of claims 1(ii) to 4(ii) and a heavy chain variable region of any one of claims 1(ii) to 4(ii).

10. A host cell or non-human host comprising:
   (i) the vector of claim 9; or
   (ii) the vector of claim 7 and the vector of claim 8, wherein these vectors comprise the nucleic acid molecules encoding for matching light chain and heavy chain variable regions as defined in claims 1(i) to 3(i), or 1(ii) to 3(ii).

11. A method for the production of an antibody that specifically binds to DDR1 which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17, the method comprising culturing the host cell of claim 10 under suitable conditions and isolating the antibody produced.

12. An antibody that specifically binds to DDR1 which is phosphorylated at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17, wherein the antibody is obtainable by the method of claim 11.

13. A composition comprising at least one of:
   (i) the antibody of any one of claims 1 to 4 or 12,
   (ii) the nucleic acid molecule of claim 5 and/or 6,
   (iii) the vector of claim 7, 8 or 9,
   (iv) the host cell of claim 10, and/or
   (v) the antibody produced by the method of claim 11.

14. Use of the antibody of any one of claims 1 to 4 or 12 for determining phosphorylation of

DDR1 at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17.

15. A method of determining phosphorylation of DDR1 at the tyrosine in position 796 of the DDR1 sequence represented in SEQ ID NO:17, the method comprising detecting the binding of the antibody of any one of claims 1 to 4 or 12 to DDR1.

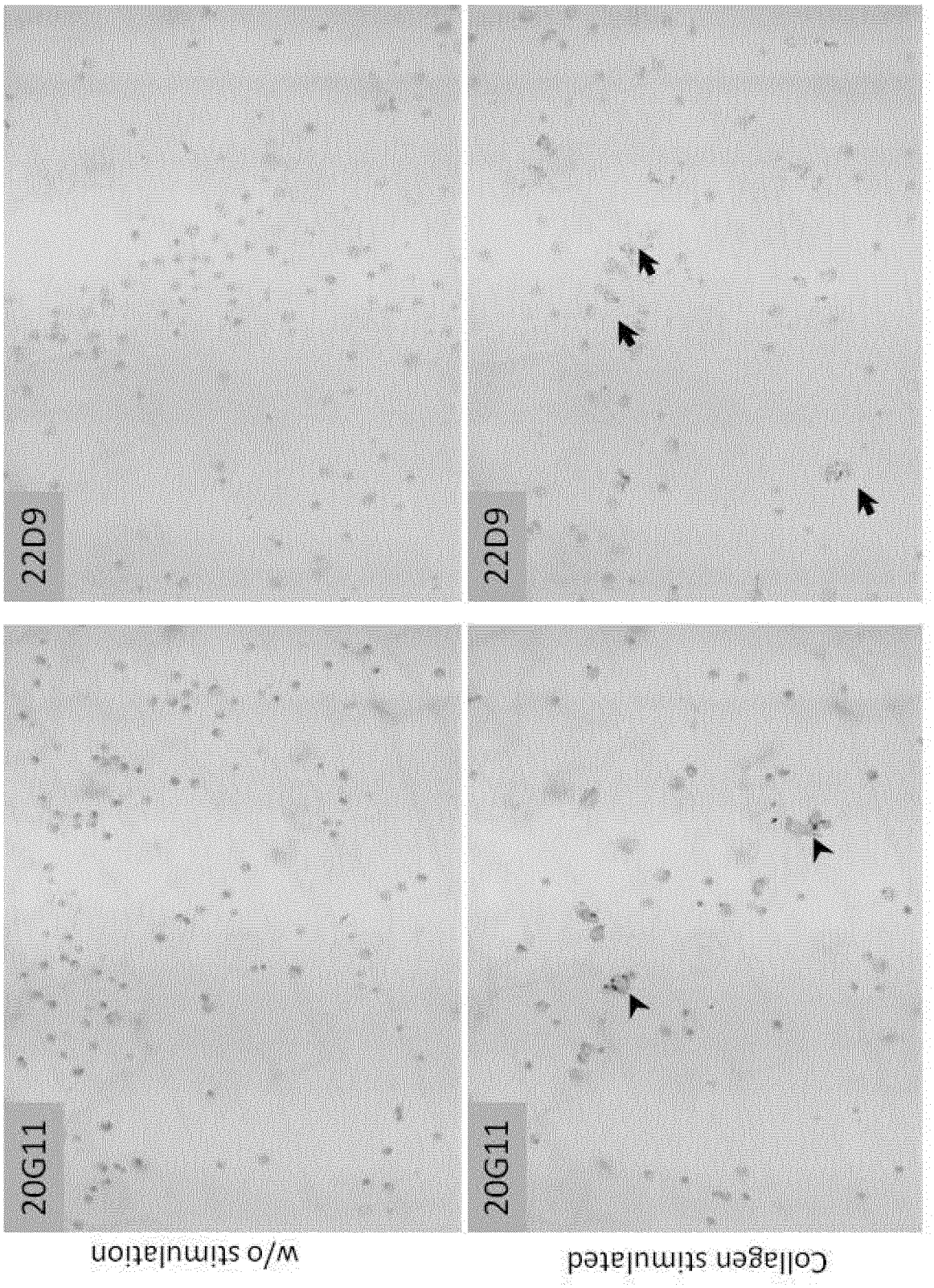

Figure 1

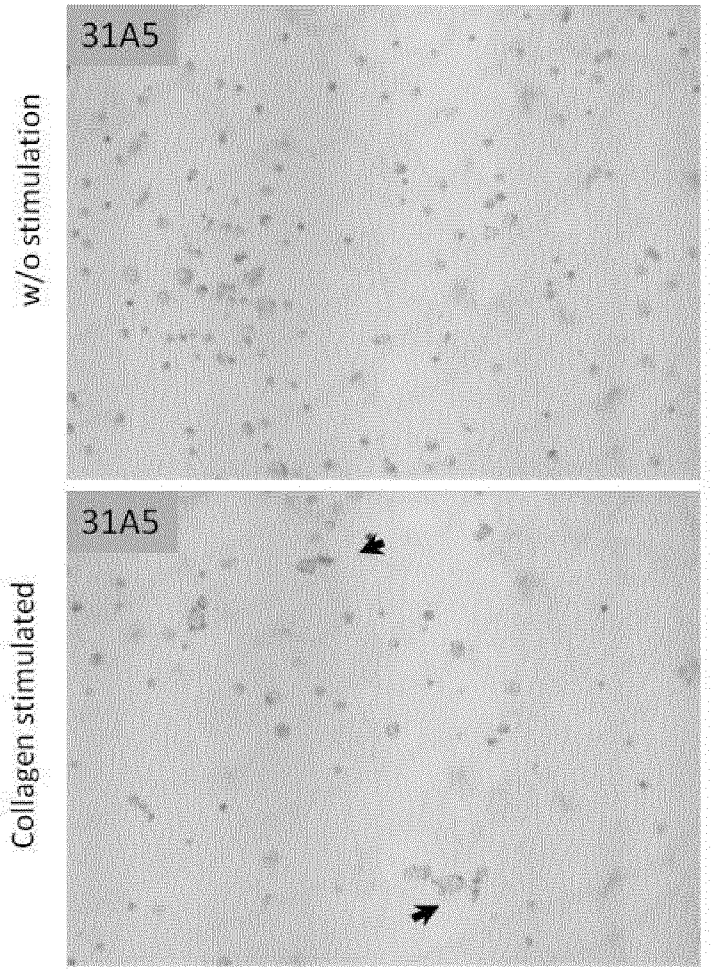

Figure 1 continued

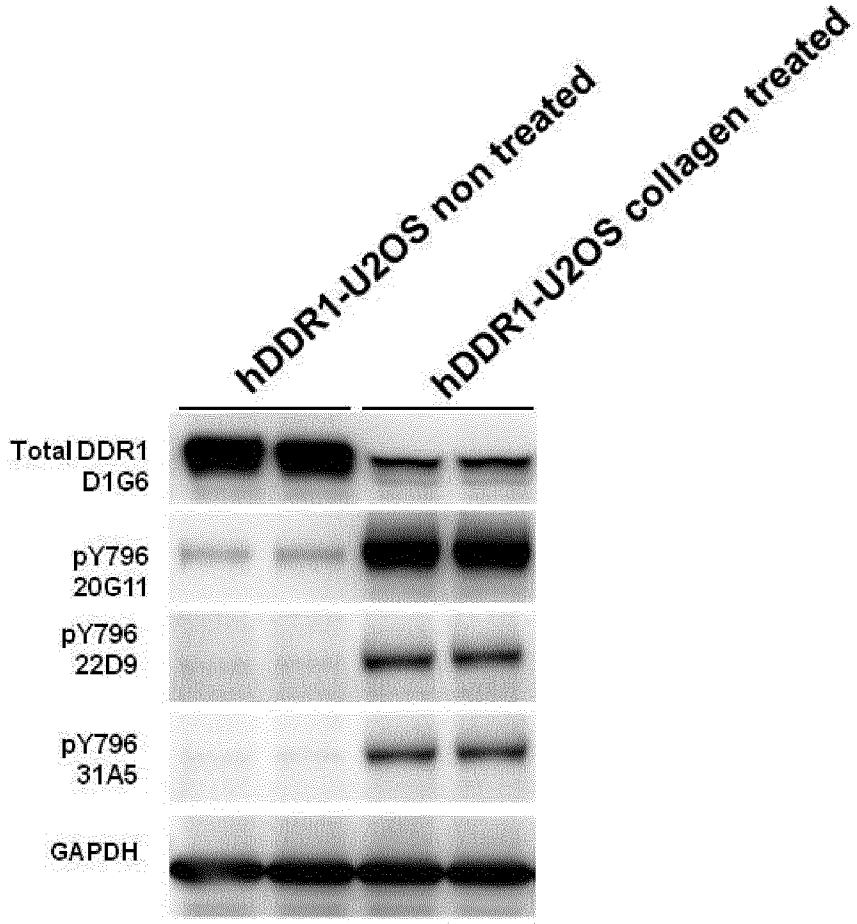
hDDR1-U2OS non treated
hDDR1-U2OS collagen treated
Total DDR1 D1G6
pY796 20G11
pY796 22D9
pY796 31A5
GAPDH

Figure 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/078186

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 749 572 A1 (CHUGAI PHARMACEUTICAL CO LTD [JP]) 2 July 2014 (2014-07-02) paragraphs [0007] - [0009], [0024], [0025], [0177] - [0179]; claims 1,4	1-15
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A	EP 2 518 157 A1 (SANOFI SA [FR]) 31 October 2012 (2012-10-31) paragraphs [0007] - [0009], [0040]	1-15

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 December 2017	23/01/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Page, Michael

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/078186

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 98/34954 A2 (MOUNT SINAI HOSPITAL CORP [CA]; VOGEL WOLFGANG [CA]; PAWSON ANTHONY [C) 13 August 1998 (1998-08-13) page 2, line 15 - page 4, line 9 page 22, lines 11-28 claim 14	1-15
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A	LEMEER SIMONE ET AL: "Phosphotyrosine mediated protein interactions of the discoidin domain receptor 1", JOURNAL OF PROTEOMICS, vol. 75, no. 12, 26 October 2011 (2011-10-26), pages 3465-3477, XP028927941, ISSN: 1874-3919, DOI: 10.1016/J.JPROT.2011.10.007 abstract page 3466, left-hand column paragraphs [03.1], [03.2]; figure 1; table 2	1-15
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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