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(54) **Title**: AMINO ACID SEQUENCES DIRECTED AGAINST DICKKOPF-1 AND POLYPEPTIDES COMPRISING THE SAME FOR THE TREATMENT OF DISEASES AND DISORDERS ASSOCIATED WITH BONE LOSS AND/OR OSTEOLYTIC LESIONS

(57) **Abstract**: Amino acid sequences are provided that are directed against Dickkopf-1 (Dkk-1), as well as compounds or constructs, and in particular proteins and polypeptides, that comprise or essentially consist of one or more such amino acid sequences. The invention also relates to compositions, and in particular to pharmaceutical compositions, that comprise such amino acid sequences, compounds, constructs and/or polypeptides, and to uses of such amino acid sequences, compounds, constructs, polypeptides, and/or compositions, for prevention and/or treatment of diseases and disorders associated with bone loss and/or osteolytic lesions.

Amino acid sequences directed against Dickkopf-1 and polypeptides comprising the same for the treatment of diseases and disorders associated with bone loss and/or osteolytic lesions

The present invention relates to amino acid sequences that are directed against (as defined herein) Dickkopf-1 (Dkk-1), as well as to compounds or constructs, and in particular proteins and polypeptides, that comprise or essentially consist of one or more such amino acid sequences (also referred to herein as "*amino acid sequences of the invention*", "*compounds of the invention*", and "*polypeptides of the invention*", respectively).

The invention also relates to nucleic acids encoding such amino acid sequences and polypeptides (also referred to herein as "*nucleic acids of the invention*" or "*nucleotide sequences of the invention*"); to methods for preparing such amino acid sequences and polypeptides; to host cells expressing or capable of expressing such amino acid sequences or polypeptides; to compositions, and in particular to pharmaceutical compositions, that comprise such amino acid sequences, polypeptides, nucleic acids and/or host cells; and to uses of such amino acid sequences or polypeptides, nucleic acids, host cells and/or compositions, in particular for prophylactic, therapeutic or diagnostic purposes, such as the prophylactic, therapeutic or diagnostic purposes mentioned herein.

Other aspects, embodiments, advantages and applications of the invention will become clear from the further description herein.

Dickkopf-1 (Dkk-1) is a member of the dickkopf family of proteins which currently contains four family members (Krupnik et al. 1999, Gene 238: 301), namely Dkk-1 (human DNA: accno. NM_012242; PRT accno. O94907), Dkk-2 (human accno. NM_014421; PRT accno. NP_055236), Dkk-3 (human accno. NM_015881; PRT accno. AAQ88744), and Dkk-4 (human accno. NM_014420; PRT accno. NP_055235). The amino acid sequence of human Dkk-1 and nucleotides encoding it have also been described in Feddi et al. (1999, J. Biol. Chem. 274: 19465), US 6,344,541, US 6,844,422, US 7,057,017, US 2005/0069915 and Krupnick et al.(1999, Gene 238: 301).

The Dkk family of proteins have been shown to be negative regulators of the canonical Wnt-signaling pathway, which has a central role in bone development and formation (Glirka et al. 1998, Nature 391: 357-62; Fedi et al. 1999, J. Biol. Chem. 274: 19465-72; Zorn 2001, Curr. Biol. Il: R592- 95; and Krupnik et al. 1999, Gene 238: 301-13). Dkk-1 inhibits Wnt signaling through its interaction with the Wnt co-receptors, low density lipoprotein receptor-related proteins (LRP)5 or 6 (LRP5/6) and the Kremen proteins (Bafico et al. 2001, Nature Cell Biol. 3: 683; Mao et al. 2001, Nature 411: 321; Mao et al. 2002, Nature 417: 664; Semenov et al. 2001, Curr. Biol. 11: 951-61). By binding LRP5 (LRP6) and Kremen proteins, Dkk-1 prevents LRP5 or LRP6 from associating with members of the Wnt pathway

and thus prevents Wnt-mediated signal transduction, which in turn results in the inhibition of bone formation. Dkk-1 possesses the ability to inhibit Wnt-induced axis duplication, and genetic analysis indicates that Dkk-1 acts upstream to inhibit Wnt signaling. Dkk-1 is also important in skeletal development as demonstrated by effects on the loss of bone structures in chicken and mouse embryos after exposure to elevated levels of Dkk-1 (Tian et al, 2003, New England J. Med. 349: 2483-2494).

LRP5 is a key protein in regulating bone mass (Gong et al. 2001, Cell 107: 513-23; Patel 2002, N. Eng. J. Med. 346: 1572). An autosomal recessive disorder characterized by low bone mass (osteoporosis- pseudoglioma syndrome, or "OPPG") has been identified as being caused by loss of-function mutations in LRP5 (Gong et al. 2001, Cell 107: 513-23). In addition, gain-of-function mutations in LRP5 have been shown to result in autosomal dominant high bone mass in humans (Little et al. 2002, Am. J. Human Genetics 70: 11-19). The same mutations in LRP5 that result in high bone mass can interfere with the ability of Dkk-1 to inhibit LRP5 signaling (Boyden et al. 2002, N. Eng. J. Med. 346: 1513-1521). Thus, Dkk-1 is appropriately characterized as being a negative regulator of bone deposition.

Dkk is composed of two characteristic cysteine-rich domains, the N-terminal (amino acids 97-138) and C-terminal (amino acids 183-245) cysteine-rich domain, respectively, each containing 10 conserved cysteines, separated by a variable-length spacer region (Krupnik et al. 1999, Gene 238: 301). Wnt antagonism by Dkk requires the binding of the C-terminal cysteine-rich domain of Dkk to the Wnt coreceptor, LRP5/6 (Semenov et al. 2001, Curr. Biol. 11: 951; Li et al. 2002, J. Biol. Chem. 277: 5977; Brott and Sokol 2002, Mol. Cell. Biol. 22: 6100; Mao and Niehrs 2003, Gene 302: 179). The Dkk-LRP5/6 complex antagonizes canonical Wnt signaling by inhibiting LRP5/6 interaction with Wnt (Li et al. 2002, J. Biol. Chem. 277: 5977; Bafico et al. 2001, Nat. Cell Biol. 3: 683; Li et al. 2004, Oncogene 23: 9129) and by forming a ternary complex with the transmembrane protein Kremen (Mao et al. 2002, Nature 417: 664; Zhang et al. 2004, Mol. Cell Biol. 24: 4677) that promotes internalization of LRP5/6.

The LRP-binding surface on Dkk-1 is centered at residues Lys^{217}, Arg^{242}, His^{210} and His^{267} (Chen et al. 2008, J. Biol. Chem. 34: 23364). Mutations at corresponding residues in Dkk2C strongly disrupted both Dkk-mediated inhibition of Wnt signaling and LRP6 binding. Dkk-1 residues Arg^{197}, Ser^{198}, and Lys^{232} are specifically involved in its binding to Kremen rather than to LRP6. These residues are localized at a surface that is at the opposite side of the LRP-binding surface. Dkk-1 mutants carrying a mutation at Arg^{197}, Ser^{198}, or Lys^{232} could antagonize Wnt signaling as well as the wild-type Dkk-1. These mutations only affected their ability to antagonize Wnt signaling when both LRP6 and Kremen were co-expressed (Wang et al. 2008, J. Biol. Chem. 283: 23371).

Elevated Dkk-1 serum levels have also been associated with prostate cancer, and elevated Dkk-1 and RANKL levels in bone marrow plasma and peripheral blood in patients with multiple myeloma have been associated with the presence of focal bone lesions. Therefore, Dkk-1 has also been implicated in the pathogenesis of myeloma bone disease through the suppression of osteoblast differentiation (Tian et al. 2003, N. Engl. J. Med. 349: 2483-94). Recent, preclinical studies have demonstrated that treatments with antibodies against Dkk-1 were sufficient to repair the bone lesions in multiple myeloma and rheumatoid arthritis and restore the damaged mucosa in experimental colitis, respectively (Zhao et al. 2008, Trends Biotechno. 27: 131). Dkk-1 as a possible target for intervention of metastatic bone lesions in breast cancer is currently being investigated in clinical trials (Zhao et al. 2008, Trends Biotechno. 27: 131).

Agents that modulate Wnt signaling could prove to be significantly beneficial in the therapeutic development of tissue repair strategies, especially for lytic bone lesions in human diseases. A rat monoclonal antibody 11H10 has been described that shows significant anabolic activity in intact and oviarectomized models in rat (WO 06/015373). A human anti-Dkk-1 antibody RH2-18 caused a significant increase in bone mass in growing female mice (WO 08/097510). BHQ880 is a fully human, anti-DKK-1 neutralizing antibody (Ettenberg et al. BHQ880, a novel anti-DKK1 neutralizing antibody, inhibits tumor-induced osteolytic bone disease. Presented at: American Association for Cancer Research Annual Meeting. April 12-16, 2008; San Diego, Calif. Abstract). Preclinical studies support the hypothesis that BHQ880 promotes bone formation and thereby inhibits tumor-induced osteolytic disease. BHQ880 is currently undergoing Phase I clinical trials (Novartis).

There is still a clear need for additional anabolic agents which rapidly return bone loss to age-matched or normal levels. There is a clear need for additional anabolic agents to reduce or prevent bone lesions associated with multiple myeloma and other malignancies (such as e.g. metastatic colon, pancreatic, breast cancers, neuroblastoma, lung cancer) and to promote tumour regression.

The present invention provides polypeptides and compositions that can generally be used to modulate, and in particular inhibit and/or prevent, binding of Dkk-1 to LRP5/6 and/or binding of Dkk-1 to Kremen, and thus to modulate, and in particular neutralize the inhibitory effect on Wnt signaling that is mediated by the Dkk-1 interaction with LRP5/6 and/or the Dkk-1 interaction with Kremen, to modulate the biological pathways in which Dkk-1, LRP5/6 and/or Kremen are involved, and/or to modulate the biological mechanisms, responses and effects associated with such signaling or these pathways.

As such, the polypeptides and compositions of the present invention can be used for the prevention and treatment (as defined herein) of Dkk-1 -associated diseases or disorders. Generally, "Dkk-1 -associated diseases or disorders" can be defined as diseases and disorders that can be prevented and/or treated, respectively, by suitably administering to a subject in need thereof (i.e. having the disease or disorder or at least one symptom thereof and/or at risk of attracting or developing the disease or disorder) of either a polypeptide or composition of the invention (and in particular, of a pharmaceutically active amount thereof) and/or of a known active principle active against Dkk-1 or a biological pathway or mechanism in which Dkk-1 is involved (and in particular, of a pharmaceutically active amount thereof). Examples of such Dkk-1 -associated diseases or disorders will be clear to the skilled person based on the disclosure herein, and for example include but are not limited to diseases or disorders that involve excessive bone loss or that require the formation of new bone such as ostopenia, osteoporosis including glucocorticoid induced osteoporosis, osteoporosis induced after transplantation, osteoporosis associated with chemotherapy, post-menopausal osteoporosis, immobilization induced osteoporosis, osteoporosis due to mechanical unloading, and osteoporosis associated with anticonvulsant use, periodontitis, Paget's disease, bone loss due to immobilization, lytic bone metastases, arthritis including osteoarthritis, rheumatoid arthritis, psoriatic-arthritis, ankylosing spondylitis and other conditions that involve bone erosion; bone disease associated with renal failure and nutritional, gastrointestinal and/or hepatic associated bone diseases; osteolytic lesions - especially osteolytic lesions associated with a myeloma, especially a multiple myeloma (Tian et al. 2003, N. Engl. J. Med. 349: 2483-94), or with cancers of the bone, breast, colon, melanocytes, hepatocytes, epithelium, esophagus, brain, lung, prostate or pancreas or metastasis thereof; bone loss associated with transplantation; osteosarcoma, prostate cancer, hepatocellular carcinoma (HCC), myeloma including multiple myeloma, diabetes, obesity, muscle wasting, Alzheimer's disease, neuroblastoma, cancers known to increase osteoclastic activity and/or induce bone resorption such as colon cancer (Fedi et al. 1999, J. Biol. Chem. 274: 19465), melanomas(Fedi et al. 1999, J. Biol. Chem. 274: 19465), hepatocellular carcinomas(Fedi et al. 1999, J. Biol. Chem. 274: 19465), prostate cancer, bone lesions in metastatic breast cancer (Zhao et al. 2008, Trends Biotechno. 27: 131); colitis and mucositis including experimental colitis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis; unwanted hair loss, initiation of hair follicle placode formation; diseases responsive to stem cell renewal including diabetes, chronic hear failure and diseases of the muscle.

In particular, the polypeptides and compositions of the present invention can be used for the prevention and treatment of diseases and disorders which are characterized by excessive and/or unwanted inhibition of Wnt signaling mediated by Dkk-1 or by the pathway(s) in which Dkk-1 is

involved. Examples of such diseases and disorders will again be clear to the skilled person based on the disclosure herein.

Thus, without being limited thereto, the amino acid sequences and polypeptides of the invention can for example be used to prevent and/or to treat all diseases and disorders that are currently being prevented or treated with active principles that can modulate Dkk-1-mediated signaling, such as those mentioned in the prior art cited above. It is also envisaged that the polypeptides of the invention can be used to prevent and/or to treat all diseases and disorders for which treatment with such active principles is currently being developed, has been proposed, or will be proposed or developed in future. In addition, it is envisaged that, because of their favourable properties as further described herein, the polypeptides of the present invention may be used for the prevention and treatment of other diseases and disorders than those for which these known active principles are being used or will be proposed or developed; and/or that the polypeptides of the present invention may provide new methods and regimens for treating the diseases and disorders described herein.

Other applications and uses of the amino acid sequences and polypeptides of the invention will become clear to the skilled person from the further disclosure herein.

Generally, it is an object of the invention to provide pharmacologically active agents, as well as compositions comprising the same, that can be used in the diagnosis, prevention and/or treatment of Dkk-1 -associated diseases or disorders and of the further diseases and disorders mentioned herein; and to provide methods for the diagnosis, prevention and/or treatment of such diseases and disorders that involve the administration and/or use of such agents and compositions.

In particular, it is an object of the invention to provide such pharmacologically active agents, compositions and/or methods that have certain advantages compared to the agents, compositions and/or methods that are currently used and/or known in the art. These advantages will become clear from the further description below.

More in particular, it is an object of the invention to provide therapeutic proteins that can be used as pharmacologically active agents, as well as compositions comprising the same, for the diagnosis, prevention and/or treatment of Dkk-1 -associated diseases or disorders and of the further diseases and disorders mentioned herein; and to provide methods for the diagnosis, prevention and/or treatment of such diseases and disorders that involve the administration and/or the use of such therapeutic proteins and compositions.

Accordingly, it is a specific object of the present invention to provide amino acid sequences that are directed against (as defined herein) Dkk-1, in particular against Dkk-1 from a warm-blooded animal, more in particular against Dkk-1 from a mammal, and especially against human Dkk-1; and to

provide proteins and polypeptides comprising or essentially consisting of at least one such amino acid sequence.

In particular, it is a specific object of the present invention to provide such amino acid sequences and such proteins and/or polypeptides that are suitable for prophylactic, therapeutic and/or diagnostic use in a warm-blooded animal, and in particular in a mammal, and more in particular in a human being.

More in particular, it is a specific object of the present invention to provide such amino acid sequences and such proteins and/or polypeptides that can be used for the prevention, treatment, alleviation and/or diagnosis of one or more diseases, disorders or conditions associated with Dkk-1 and/or mediated by Dkk-1 (such as the diseases, disorders and conditions mentioned herein) in a warm-blooded animal, in particular in a mammal, and more in particular in a human being.

It is also a specific object of the invention to provide such amino acid sequences and such proteins and/or polypeptides that can be used in the preparation of pharmaceutical or veterinary compositions for the prevention and/or treatment of one or more diseases, disorders or conditions associated with and/or mediated by Dkk-1 (such as the diseases, disorders and conditions mentioned herein) in a warm-blooded animal, in particular in a mammal, and more in particular in a human being.

In the invention, generally, these objects are achieved by the use of the amino acid sequences, proteins, polypeptides and compositions that are described herein.

In general, the invention provides amino acid sequences that are directed against (as defined herein) and/or can specifically bind (as defined herein) to Dkk-1; as well as compounds and constructs, and in particular proteins and polypeptides, that comprise at least one such amino acid sequence.

More in particular, the invention provides amino acid sequences that can bind to Dkk-1 with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein; as well as compounds and constructs, and in particular proteins and polypeptides, that comprise at least one such amino acid sequence.

In particular, amino acid sequences and polypeptides of the invention are preferably such that they:

- bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles);

and/or such that they:

- bind to Dkk-1 with a k_{on}-rate of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$;

and/or such that they:

- bind to Dkk-1 with a k_{off} rate between 1 s^{-1} ($t_{1/2}$=0.69 s) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Preferably, a monovalent amino acid sequence of the invention (or a polypeptide that contains only one amino acid sequence of the invention) is preferably such that it will bind to Dkk-1 with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

Some preferred IC50 values for binding of the amino acid sequences or polypeptides of the invention to Dkk-1 will become clear from the further description and examples herein.

For binding to Dkk-1, an amino acid sequence of the invention will usually contain within its amino acid sequence one or more amino acid residues or one or more stretches of amino acid residues (i.e. with each "stretch" comprising two or amino acid residues that are adjacent to each other or in close proximity to each other, i.e. in the primary or tertiary structure of the amino acid sequence) via which the amino acid sequence of the invention can bind to Dkk-1, which amino acid residues or stretches of amino acid residues thus form the "site" for binding to Dkk-1 (also referred to herein as the "*antigen binding site*").

The amino acid sequences provided by the invention are preferably in essentially isolated form (as defined herein), or form part of a protein or polypeptide of the invention (as defined herein), which may comprise or essentially consist of one or more amino acid sequences of the invention and which may optionally further comprise one or more further amino acid sequences (all optionally linked via one or more suitable linkers). For example, and without limitation, the one or more amino acid sequences of the invention may be used as a binding unit in such a protein or polypeptide, which may optionally contain one or more further amino acid sequences that can serve as a binding unit (i.e. against one or more other targets than Dkk-1), so as to provide a monovalent, multivalent or multispecific polypeptide of the invention, respectively, all as described herein. Such a protein or polypeptide may also be in essentially isolated form (as defined herein).

The amino acid sequences and polypeptides of the invention as such preferably essentially consist of a single amino acid chain that is not linked via disulphide bridges to any other amino acid sequence or chain (but that may or may not contain one or more intramolecular disulphide bridges.

For example, it is known that Nanobodies® – as described herein - may sometimes contain a disulphide bridge between CDR3 and CDR1 or FR2). However, it should be noted that one or more amino acid sequences of the invention may be linked to each other and/or to other amino acid sequences (e.g. via disulphide bridges) to provide peptide constructs that may also be useful in the invention (for example Fab' fragments, $F(ab')_2$ fragments, ScFv constructs, "diabodies" and other multispecific constructs. Reference is for example made to the review by Holliger and Hudson, Nat Biotechnol. 2005 Sep;23(9):1126-36).

Generally, when an amino acid sequence of the invention (or a compound, construct or polypeptide comprising the same) is intended for administration to a subject (for example for therapeutic and/or diagnostic purposes as described herein), it is preferably either an amino acid sequence that does not occur naturally in said subject; or, when it does occur naturally in said subject, in essentially isolated form (as defined herein).

It will also be clear to the skilled person that for pharmaceutical use, the amino acid sequences of the invention (as well as compounds, constructs and polypeptides comprising the same) are preferably directed against human Dkk-1; whereas for veterinary purposes, the amino acid sequences and polypeptides of the invention are preferably directed against Dkk-1 from the species to be treated, or at at least cross-reactive with Dkk-1 from the species to be treated.

Furthermore, an amino acid sequence of the invention may optionally, and in addition to the at least one binding site for binding against Dkk-1, contain one or more further binding sites for binding against other antigens, proteins or targets.

The efficacy of the amino acid sequences and polypeptides of the invention, and of compositions comprising the same, can be tested using any suitable in vitro assay, cell-based assay, in vivo assay and/or animal model known per se, or any combination thereof, depending on the specific disease or disorder involved. Suitable assays and animal models will be clear to the skilled person, and for example include Biacore, ELISA,ELISA based competition assay, Time-resolved fluorescent (TRF) cell-based assay (WO 08/097510), TCF/Lef luciferase assay (WO 06/015373, WO 07/084344; WO 08/097510; van Bezooijen RL et al. 2007, J. Bone Miner. Res. 22: 19-28), ST2 assay (mouse stromal cell line; ALP readout) (WO 06/015373), Osteoblastic cell differentiation of mesenchymal pluripotent cell line (e.g. C3H10T1/2) (WO 08/097510), TOPFlash transcription assay (WO 08/097510), Inhibition of canonical signaling in mouse osteogenic KS483 cell line, intact mice models in which BMD is measured (WO 06/015373), Synergeneic mouse model (WO 07/084344), Oviarectomized mice, Mice engrafted with multiple myeloma cells expressing various levels of Dkk-1 (Yaccoby et al. 2007, Blood 109: 2106), Osteolytic prostate tumor model (WO 07/084344; Kim et al. 2003, Clin. Cancer Res. 9: 1200), SCID mice of human colon cancer xenografts (Lozupone et al. 2004,

Cancer Research 64: 378–385), SCID mice model for multiple myeloma (Cosette 1998, Blood, 91: 4727-4737), SCID-rab model (Yata and Yaccoby 2004, Leukemia. 18: 1891-7), SCID-hu mice model (McCune, et al. 1988, Science 241: 1632-9; Colorado State University, Fort Collins, Colo.; Jackson Laboratory, Bar Harbor, Me., Charles River Laboratories, Wilmington, Mass.), 5TGM1 mouse model (Dian et al. 2005, Mol. Cancer Ther. 4: 91), as well as the assays and animal models used in the experimental part below and in the prior art cited herein.

Also, according to the invention, amino acid sequences and polypeptides that are directed against Dkk-1 from a first species of warm-blooded animal may or may not show cross-reactivity with Dkk-1 from one or more other species of warm-blooded animal. For example, amino acid sequences and polypeptides directed against human Dkk-1 may or may not show cross reactivity with Dkk-1 from one or more other species of primates (such as, without limitation, monkeys from the genus *Macaca* (such as, and in particular, cynomolgus monkeys (*Macaca fascicularis*) and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*)) and/or with Dkk-1 from one or more species of animals that are often used in animal models for diseases (for example mouse, rat, rabbit, pig or dog), and in particular in animal models for diseases and disorders associated with Dkk-1 (such as the species and animal models mentioned herein). In this respect, it will be clear to the skilled person that such cross-reactivity, when present, may have advantages from a drug development point of view, since it allows the amino acid sequences and polypeptides against human Dkk-1 to be tested in such disease models. Preferably, the amino acid sequences and polypeptides of the invention are at least cross reactive with human Dkk-1 and mouse Dkk-1; or with human Dkk-1 and cynomolgus Dkk-1. In a preferred aspect the amino acid sequences and polypeptides of the invention are cross-reactive with human Dkk-1, mouse Dkk-1 and cynomolgus monkey Dkk-1.

More generally, amino acid sequences and polypeptides of the invention that are cross-reactive with Dkk-1 from multiple species of mammal will usually be advantageous for use in veterinary applications, since it will allow the same amino acid sequence or polypeptide to be used across multiple species. Thus, it is also encompassed within the scope of the invention that amino acid sequences and polypeptides directed against Dkk-1 from one species of animal (such as amino acid sequences and polypeptides against human Dkk-1) can be used in the treatment of another species of animal, as long as the use of the amino acid sequences and/or polypeptides provide the desired effects in the species to be treated.

The present invention is in its broadest sense also not particularly limited to or defined by a specific antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of Dkk-1 against which the amino acid sequences and polypeptides of the invention are directed. For

example, the amino acid sequences and polypeptides may or may not be directed against an "interaction site" (as defined herein).However, it is generally assumed and preferred that the amino acid sequences and polypeptides of the invention are preferably directed against an interaction site (as defined herein).On one aspect, the amino acid sequences and polypeptides of the invention are directed against the N-terminal cysteine-rich domain (amino acids 97-138). In another aspect, the amino acid sequences and polypeptides of the invention are directed against the C-terminal cysteine-rich domain (amino acids 183-245), and in particular against the LRP5/6 binding site on Dkk-1 (preferably against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267, preferably against at least one of amino acid residues R242 or H210; Chen et al. 2008, J. Biol. Chem. 34: 23364) and/or capable of competing with LRP5/6 for binding to Dkk-1. Thus, in one preferred, but non-limiting aspect, the amino acid sequences and polypeptides of the invention are directed against the LRP5/6 binding site onDkk-1 and/or capable of competing with LRP5/6 for binding to Dkk-1, and are as further defined herein.

In another aspect, the amino acid sequences and polypeptides of the invention may also be directed against the BHQ880 binding site on Dkk-1 and/or capable of competing with BHQ880 for binding to Dkk-1. Thus, in one preferred, but non-limiting aspect, the amino acid sequences and polypeptides of the invention are directed against the BHQ880 binding site onDkk-1 and/or capable of competing with BHQ880 for binding to Dkk-1, and are as further defined herein.

In yet another aspect, the amino acid sequences and polypeptides of the invention may also be directed against the RH2-18 binding site on Dkk-1 and/or capable of competing with RH2-18 for binding to Dkk-1. Thus, in one preferred, but non-limiting aspect, the amino acid sequences and polypeptides of the invention are directed against the RH2-18 binding site on Dkk-1 and/or capable of competing with RH2-18 for binding to Dkk-1, and are as further defined herein.

In yet another aspect, the amino acid sequences and polypeptides of the invention may also be directed against the 11H10 binding site on Dkk-1 and/or capable of competing with 11H10 for binding to Dkk-1. Thus, in one preferred, but non-limiting aspect, the amino acid sequences and polypeptides of the invention are directed against the 11H10 binding site onDkk-1 and/or capable of competing with 11H10 for binding to Dkk-1, and are as further defined herein.

In yet another aspect, the amino acid sequences and polypeptides of the invention may also be directed against the Kremen binding site on Dkk-1 (preferably against at least one of amino acid residues R197, R209, K214, or K232, preferably against at least one of amino acid residues R197 or K232 (Wang et al. 2008, J. Biol. Chem. 283: 23371)) and/or capable of competing with Kremen for binding to Dkk-1. Thus, in one preferred, but non-limiting aspect, the amino acid sequences and

polypeptides of the invention are directed against the Kremen binding site onDkk-1 and/or capable of competing with Kremen for binding to Dkk-1, and are as further defined herein.

As further described herein, a polypeptide of the invention may contain two or more amino acid sequences of the invention that are directed against Dkk-1. Generally, such polypeptides will bind to Dkk-1 with increased avidity compared to a single amino acid sequence of the invention. Such a polypeptide may for example comprise two amino acid sequences of the invention that are directed against the same antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of Dkk-1 (which may or may not be an interaction site); or comprise at least one "first" amino acid sequence of the invention that is directed against a first same antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of Dkk-1 (which may or may not be an interaction site); and at least one "second" amino acid sequence of the invention that is directed against a second antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) different from the first (and which again may or may not be an interaction site). Preferably, in such "biparatopic" polypeptides of the invention, at least one amino acid sequence of the invention is directed against an interaction site (as defined herein), although the invention in its broadest sense is not limited thereto.

In a preferred aspect, the amino acid sequences and (in particular) polypeptides of the invention are capable of binding to two or more different antigenic determinants, epitopes, parts, domains of Dkk-1. In this context, the amino acid sequences and polypeptides of the invention are also referred to as "multiparatopic" (such as e.g. "biparatopic" or "triparatopic", etc.) amino acid sequences and polypeptides. The multiparatopic amino acid sequences and polypeptides of the invention can be directed against any antigenic determinants, epitopes, parts, and/or domains of Dkk-1.

For example, and generally, a biparatopic polypeptide of the invention may comprise at least one amino acid sequence of the invention directed against a first antigenic determinant, epitope, part or domain of Dkk-1 and at least one amino acid sequence of the invention directed against a second antigenic determinant, epitope, part or domain of Dkk-1 different from the first antigenic determinant, epitope, part or domain (in which said amino acid sequences may be suitably linked, for example via a suitable linker as further described herein). Preferably, such a biparatopic polypeptide of the invention is further such that, when it binds to Dkk-1, it is capable of simultaneously binding to the first antigenic determinant, epitope, part or domain (i.e. via the at least one amino acid sequence of the invention capable of binding to said first antigenic determinant, epitope, part or domain) and binding to said second antigenic determinant, epitope, part or domain (i.e. via the at least one amino acid sequence of the invention capable of binding to

said second antigenic determinant, epitope, part or domain). Examples of such biparatopic polypeptides of the invention will become clear from the further description herein. Also, a triparatopic polypeptide of the invention may comprise at least one further amino acid sequence of the invention directed against a third antigenic determinant, epitope, part or domain of Dkk-1 (different from both the first and second antigenic determinant, epitope, part or domain), and generally multiparatopic polypeptides of the invention may contain at least two amino acid sequences of the invention directed against at least two different antigenic determinants, epitopes, parts or domains of Dkk-1. Generally, such biparatopic, triparatopic and multiparatopic polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic, triparatopic and multiparatopic polypeptides of the invention (for example, these biparatopic, triparatopic and multiparatopic polypeptides of the invention preferably comprise single variable domains and more preferably Nanobodies®).

In a preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic (or multiparatopic) and are directed against the LRP5/6 binding site on Dkk-1 and/or capable of competing with LRP5/6 for binding to Dkk-1, as well as against at least one other antigenic determinant, epitope, part or domain on Dkk-1. The amino acid sequences and polypeptides of the invention may bind at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1 as well as at least one other antigenic determinant, epitope, part or domain on Dkk-1. In a preferred aspect, the amino acid sequences and polypeptides of the invention bind amino acid residues R242 or H210 of Dkk-1 as well as at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such a biparatopic (or multiparatopic) polypeptide of the invention will contain at least one amino acid sequence of the invention that is capable of binding to the LRP5/6 binding site on Dkk-1 and/or capable of competing with LRP5/6 for binding to Dkk-1 (and in particular to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1 and more preferably to at least one of amino acid residues R242 or H210 of Dkk-1), as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the LRP5/6 binding site and the at least one other

antigenic determinant, epitope, part or domain on Dkk-1; and preferably comprise single variable domains and more preferably Nanobodies®).

In another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic (or multiparatopic) and are at least capable, upon binding to Dkk-1, to modulate (the inhibitory function of Dkk-1 on) Wnt-mediated signal transduction (i.e. the signaling that is caused by binding of LRP5/6 to members of the Wnt pathway) and/or to modulate (neutralize) the inhibitory function of Dkk-1 on osteoblastic cell differentiation.

Such biparatopic (or multiparatopic) polypeptides of the invention preferably comprise at least one amino acid sequence of the invention that is capable, upon binding to Dkk-1, to modulate (the inhibitory effect of Dkk-1 on) Wnt-mediated signal transduction (the Wnt/beta-catenin signaling pathway) and/or to modulate (neutralize) the inhibitory function of Dkk-1 on osteoblastic cell differentiation; as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1 (i.e. different from the antigenic determinant, epitope, part or domain to which the aforementioned amino acid sequence of the invention can bind). Such biparatopic (or multiparatopic) polypeptides of the invention preferably comprise at least one amino acid sequence of the invention that is directed against the LRP5/6 binding site on Dkk-1 and/or capable of competing with LRP5/6 for binding to Dkk-1 (and in particular against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1 and more preferably to against at least one of amino acid residues R242 or H210 of Dkk-1), as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the LRP5/6 binding site and the at least one other antigenic determinant, epitope, part or domain on Dkk-1; and preferably comprise single variable domains and more preferably Nanobodies®).

In another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic (or multiparatopic) and are directed against the BHQ880 binding site on Dkk-1 and/or capable of competing with BHQ880 for binding to Dkk-1, as well as against at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such a biparatopic (or multiparatopic) polypeptide of the invention will contain at least one amino acid sequence of the invention that is capable of binding to the BHQ880 binding site on

Dkk-1 and/or capable of competing with BHQ880 for binding to Dkk-1, as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the BHQ880 binding site and the at least one other antigenic determinant, epitope, part or domain on Dkk-1; and preferably comprise single variable domains and more preferably Nanobodies®).

In another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic (or multiparatopic) and are at least capable, upon binding to Dkk-1, to modulate (the inhibitory function of Dkk-1 on) Wnt-mediated signal transduction (i.e. the signaling that is caused by binding of LRP5/6 to members of the Wnt pathway) and/or to modulate (neutralize) the inhibitory function of Dkk-1 on osteoblastic cell differentiation.

Such biparatopic (or multiparatopic) polypeptides of the invention preferably comprise at least one amino acid sequence of the invention that is capable, upon binding to Dkk-1, to modulate (the inhibitory effect of Dkk-1 on) Wnt-mediated signal transduction (the Wnt/beta-catenin signaling pathway) and/or to modulate (neutralize) the inhibitory function of Dkk-1 on osteoblastic cell differentiation; as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1 (i.e. different from the antigenic determinant, epitope, part or domain to which the aforementioned amino acid sequence of the invention can bind). Such biparatopic (or multiparatopic) polypeptides of the invention preferably comprise at least one amino acid sequence of the invention that is directed against the BHQ880 binding site on Dkk-1 and/or capable of competing with BHQ880 for binding to Dkk-1, as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the BHQ880 binding site and the at least one other antigenic determinant, epitope, part or domain on Dkk-1; and preferably comprise single variable domains and more preferably Nanobodies®).

In yet another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic (or multiparatopic) and are directed against the RH2-18 binding site on Dkk-1 and/or capable of competing with RH2-18 for binding to Dkk-1, as well as against at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such a biparatopic (or multiparatopic) polypeptide of the invention will contain at least one amino acid sequence of the invention that is capable of binding to the RH2-18 binding site on Dkk-1 and/or capable of competing with RH2-18 for binding to Dkk-1, as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the RH2-18 binding site and the at least one other antigenic determinant, epitope, part or domain on Dkk-1; and preferably comprise single variable domains and more preferably Nanobodies®).

In another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic (or multiparatopic) and are at least capable, upon binding to Dkk-1, to modulate (the inhibitory function of Dkk-1 on) Wnt-mediated signal transduction (i.e. the signaling that is caused by binding of LRP5/6 to members of the Wnt pathway) and/or to modulate (neutralize) the inhibitory function of Dkk-1 on osteoblastic cell differentiation.

Such biparatopic (or multiparatopic) polypeptides of the invention preferably comprise at least one amino acid sequence of the invention that is capable, upon binding to Dkk-1, to modulate (the inhibitory effect of Dkk-1 on) Wnt-mediated signal transduction (the Wnt/beta-catenin signaling pathway) and/or to modulate (neutralize) the inhibitory function of Dkk-1 on osteoblastic cell differentiation; as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1 (i.e. different from the antigenic determinant, epitope, part or domain to which the aforementioned amino acid sequence of the invention can bind). Such biparatopic (or multiparatopic) polypeptides of the invention preferably comprise at least one amino acid sequence of the invention that is directed against the RH2-18 binding site on Dkk-1 and/or capable of competing with RH2-18 for binding to Dkk-1, as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these

biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the RH2-18 binding site and the at least one other antigenic determinant, epitope, part or domain on Dkk-1; and preferably comprise single variable domains and more preferably Nanobodies®).

In yet another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic (or multiparatopic) and are directed against the 11H10 binding site on Dkk-1 and/or capable of competing with 11H10 for binding to Dkk-1, as well as against at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such a biparatopic (or multiparatopic) polypeptide of the invention will contain at least one amino acid sequence of the invention that is capable of binding to the 11H10 binding site on Dkk-1 and/or capable of competing with 11H10 for binding to Dkk-1, as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the 11H10 binding site and the at least one other antigenic determinant, epitope, part or domain on Dkk-1; and preferably comprise single variable domains and more preferably Nanobodies®).

In another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic (or multiparatopic) and are at least capable, upon binding to Dkk-1, to modulate (the inhibitory function of Dkk-1 on) Wnt-mediated signal transduction (i.e. the signaling that is caused by binding of LRP5/6 to members of the Wnt pathway) and/or to modulate (neutralize) the inhibitory function of Dkk-1 on osteoblastic cell differentiation.

Such biparatopic (or multiparatopic) polypeptides of the invention preferably comprise at least one amino acid sequence of the invention that is capable, upon binding to Dkk-1, to modulate (the inhibitory effect of Dkk-1 on) Wnt-mediated signal transduction (the Wnt/beta-catenin signaling pathway) and/or to modulate (neutralize) the inhibitory function of Dkk-1 on osteoblastic cell differentiation; as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1 (i.e. different from the antigenic determinant, epitope, part or domain to which the aforementioned amino acid sequence of the invention can bind). Such biparatopic (or multiparatopic) polypeptides of the invention preferably comprise at least one amino acid sequence of the invention that is directed

against the 11H10 binding site on Dkk-1 and/or capable of competing with 11H10 for binding to Dkk-1, as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the 11H10 binding site and the at least one other antigenic determinant, epitope, part or domain on Dkk-1; and preferably comprise single variable domains and more preferably Nanobodies®).

In yet another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic (or multiparatopic) and are directed against the Kremen binding site on Dkk-1 and/or capable of competing with Kremen for binding to Dkk-1, as well as against at least one other antigenic determinant, epitope, part or domain on Dkk-1. The amino acid sequences and polypeptides of the invention may bind at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1 as well as at least one other antigenic determinant, epitope, part or domain on Dkk-1. In a preferred aspect, the amino acid sequences and polypeptides of the invention bind at least one of amino acid residues R197 or K232 of Dkk-1 as well as at least one other antigenic determinant , epitope, part or domain on Dkk-1. Generally, such a biparatopic (or multiparatopic) polypeptide of the invention will contain at least one amino acid sequence of the invention that is capable of binding to the Kremen binding site on Dkk-1 and/or capable of competing with Kremen for binding to Dkk-1 (and in particular against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1 and more preferably against at least one of amino acid residues R197 or K232 of Dkk-1), as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the Kremen binding site and the at least one other antigenic determinant, epitope, part or domain on Dkk-1; and preferably comprise single variable domains and more preferably Nanobodies®).

In another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic (or multiparatopic) and are at least capable, upon

binding to Dkk-1, to modulate (the inhibitory function of Dkk-1 on) Wnt-mediated signal transduction (i.e. the signaling that is caused by binding of LRP5/6 to members of the Wnt pathway) and/or to modulate (neutralize) the inhibitory function of Dkk-1 on osteoblastic cell differentiation.

Such biparatopic (or multiparatopic) polypeptides of the invention preferably comprise at least one amino acid sequence of the invention that is capable, upon binding to Dkk-1, to modulate (the inhibitory effect of Dkk-1 on) Wnt-mediated signal transduction (the Wnt/beta-catenin signaling pathway) and/or to modulate (neutralize) the inhibitory function of Dkk-1 on osteoblastic cell differentiation; as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1 (i.e. different from the antigenic determinant, epitope, part or domain to which the aforementioned amino acid sequence of the invention can bind). Such biparatopic (or multiparatopic) polypeptides of the invention preferably comprise at least one amino acid sequence of the invention that is directed against the Kremen binding site on Dkk-1 and/or capable of competing with Kremen for binding to Dkk-1 (and in particular against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1 and more preferably against at least one of amino acid residues R197 or K232 of Dkk-1), as well as at least one further amino acid sequence of the invention that is capable of binding to at least one other antigenic determinant, epitope, part or domain on Dkk-1. Generally, such biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the Kremen binding site and the at least one other antigenic determinant, epitope, part or domain on Dkk-1; and preferably comprise single variable domains and more preferably Nanobodies®).

In yet another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic and are at least directed against the LRP5/6 binding site on Dkk-1 as well as against the Kremen binding site on Dkk-1. The amino acid sequences and polypeptides of the invention may be directed against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1. The amino acid sequences and polypeptides of the invention may be directed against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1. The amino acid sequences and polypeptides of the invention may be directed against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1 as well as against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1. In a preferred aspect, the amino acid sequences and polypeptides of the invention may be directed against at least on of the

amino acids R242 or H210 of Dkk-1. In yet another preferred aspect, the amino acid sequences and polypeptides of the invention may be directed against at least on of the amino acids R242 or H210 of Dkk-1 as well as against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1. In yet another preferred aspect, the amino acid sequences and polypeptides of the invention are directed against at least one of the amino acids R197 or K232 of Dkk-1. In another preferred aspect, the amino acid sequences and polypeptides of the invention are directed against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1 as well as against at least one of the amino acids R197 or K232 of Dkk-1. In yet another preferred aspect, the amino acid sequences and polypeptides of the invention are directed against at least on of the amino acids R242 or H210 of Dkk-1 as well as against at least one of the amino acids R197 or K232 of Dkk-1.

Again, the above biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the LRP5/6 binding site and the Kremen binding site; and preferably comprise single variable domains and more preferably Nanobodies®).

In yet another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic and are at least directed against the BHQ880 binding site on Dkk-1 as well as against the Kremen binding site on Dkk-1. The amino acid sequences and polypeptides of the invention may be directed against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1. In a preferred aspect, the amino acid sequences and polypeptides of the invention may be directed against at least one of the amino acids R197 or K232 of Dkk-1.

Again, the above biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the BHQ880 binding site and the Kremen binding site; and preferably comprise single variable domains and more preferably Nanobodies®).

In yet another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic and are at least directed against the RH2-18 binding site on Dkk-1 as well as against the Kremen binding site on Dkk-1. The amino acid sequences and polypeptides of the invention may be directed against at least one of amino acid residues R197,

R209, K214, or K232 of Dkk-1. In a preferred aspect, the amino acid sequences and polypeptides of the invention may be directed against at least one of the amino acids R197 or K232 of Dkk-1.

Again, the above biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the RH2-18 binding site and the Kremen binding site; and preferably comprise single variable domains and more preferably Nanobodies®).

In yet another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic and are at least directed against the 11H10 binding site on Dkk-1 as well as against the Kremen binding site on Dkk-1. The amino acid sequences and polypeptides of the invention may be directed against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1. In a preferred aspect, the amino acid sequences and polypeptides of the invention may be directed against at least one of the amino acids R197 or K232 of Dkk-1.

Again, the above biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind the 11H10 binding site and the Kremen binding site; and preferably comprise single variable domains and more preferably Nanobodies®).

In yet another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic with both paratopes directed against the LRP5/6 binding site on Dkk-1. The amino acid sequences and polypeptides of the invention may be directed against at least one of amino acid residues W212, R242, K217, R209, H210, L266 on Dkk-1 (one paratope or both paratopes). In a preferred aspect, the amino acid sequences and polypeptides of the invention may be directed against at least one of amino acid residues R242 or H210 (one paratope or both paratopes).

In another preferred, but non-limiting aspect, the amino acid sequences and (in particular) polypeptides of the invention are biparatopic with both paratopes directed against the Kremen binding site on Dkk-1 The amino acid sequences and polypeptides of the invention may be directed against at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1 (one paratope or both paratopes). In a preferred aspect, the amino acid sequences and polypeptides of the invention may be directed against at least one of amino acid residues R197 or K232 on Dkk-1 (one paratope or both paratopes).

Again, the above biparatopic (or multiparatopic) polypeptides of the invention may be as further described herein, and the various preferred aspects of the invention as described herein also apply to these biparatopic (or multiparatopic) polypeptides of the invention (for example, these biparatopic and multiparatopic polypeptides of the invention may comprise suitable linkers; are preferably such that they can simultaneously bind both binding sites; and preferably comprise single variable domains and more preferably Nanobodies®).Also, when the target is part of a binding pair (for example, a receptor-ligand binding pair), the amino acid sequences and polypeptides may be such that they compete with the cognate binding partner (e.g. the ligand, receptor or other binding partner, as applicable) for binding to the target, and/or such that they (fully or partially) neutralize binding of the binding partner to the target.

It is also within the scope of the invention that, where applicable, an amino acid sequence of the invention can bind to two or more antigenic determinants, epitopes, parts, domains, subunits or confirmations of Dkk-1. In such a case, the antigenic determinants, epitopes, parts, domains or subunits of Dkk-1 to which the amino acid sequences and/or polypeptides of the invention bind may be essentially the same (for example, if Dkk-1 contains repeated structural motifs or occurs in a multimeric form) or may be different (and in the latter case, the amino acid sequences and polypeptides of the invention may bind to such different antigenic determinants, epitopes, parts, domains, subunits of Dkk-1 with an affinity and/or specificity which may be the same or different). Also, for example, when Dkk-1 exists in an activated conformation and in an inactive conformation, the amino acid sequences and polypeptides of the invention may bind to either one of these confirmation, or may bind to both these confirmations (i.e. with an affinity and/or specificity which may be the same or different). Also, for example, the amino acid sequences and polypeptides of the invention may bind to a conformation of Dkk-1 in which it is bound to a pertinent ligand, may bind to a conformation of Dkk-1 in which it not bound to a pertinent ligand, or may bind to both such conformations (again with an affinity and/or specificity which may be the same or different).

It is also within the scope of the invention that an amino acid sequence of the invention can bind Dkk-1 in glycosylated and/or non-glycosylated form. In one aspect, the amino acid sequence of the invention can bind glycosylated DKk-1 while not binding to non-glycosylated Dkk-1. In another aspect, the amino acid sequence of the invention can bind glycosylated DKk-1 and non-glycosylated Dkk-1. In yet another aspect, the amino acid sequence of the invention can bind non-glycosylated DKk-1 while not binding to glycosylated Dkk-1.

It is also expected that the amino acid sequences and polypeptides of the invention will generally bind to all naturally occurring or synthetic analogs, variants, mutants, alleles, parts and fragments of Dkk-1; or at least to those analogs, variants, mutants, alleles, parts and fragments of

Dkk-1 that contain one or more antigenic determinants or epitopes that are essentially the same as the antigenic determinant(s) or epitope(s) to which the amino acid sequences and polypeptides of the invention bind in Dkk-1 (e.g. in wild-type Dkk-1). Again, in such a case, the amino acid sequences and polypeptides of the invention may bind to such analogs, variants, mutants, alleles, parts and fragments with an affinity and/or specificity that are the same as, or that are different from (i.e. higher than or lower than), the affinity and specificity with which the amino acid sequences of the invention bind to (wild-type) Dkk-1. It is also included within the scope of the invention that the amino acid sequences and polypeptides of the invention bind to some analogs, variants, mutants, alleles, parts and fragments of Dkk-1, but not to others.

When Dkk-1 exists in a monomeric form and in one or more multimeric forms, it is within the scope of the invention that the amino acid sequences and polypeptides of the invention only bind to Dkk-1 in monomeric form, only bind to Dkk-1 in multimeric form, or bind to both the monomeric and the multimeric form. Again, in such a case, the amino acid sequences and polypeptides of the invention may bind to the monomeric form with an affinity and/or specificity that are the same as, or that are different from (i.e. higher than or lower than), the affinity and specificity with which the amino acid sequences of the invention bind to the multimeric form.

Also, when Dkk-1 can associate with other proteins or polypeptides to form protein complexes (e.g. with multiple subunits), it is within the scope of the invention that the amino acid sequences and polypeptides of the invention bind to Dkk-1 in its non-associated state, bind to Dkk-1 in its associated state, or bind to both. In all these cases, the amino acid sequences and polypeptides of the invention may bind to such multimers or associated protein complexes with an affinity and/or specificity that may be the same as or different from (i.e. higher than or lower than) the affinity and/or specificity with which the amino acid sequences and polypeptides of the invention bind to Dkk-1 in its monomeric and non-associated state.

Also, as will be clear to the skilled person, proteins or polypeptides that contain two or more amino acid sequences directed against Dkk-1 may bind with higher avidity to Dkk-1 than the corresponding monomeric amino acid sequence(s). For example, and without limitation, proteins or polypeptides that contain two or more amino acid sequences directed against different epitopes of Dkk-1 may (and usually will) bind with higher avidity than each of the different monomers, and proteins or polypeptides that contain two or more amino acid sequences directed against Dkk-1 may (and usually will) bind also with higher avidity to a multimer of Dkk-1.

Generally, amino acid sequences and polypeptides of the invention will at least bind to those forms of Dkk-1 (including monomeric, multimeric and associated forms) that are the most relevant from a biological and/or therapeutic point of view, as will be clear to the skilled person.

It is also within the scope of the invention to use parts, fragments, analogs, mutants, variants, alleles and/or derivatives of the amino acid sequences and polypeptides of the invention, and/or to use proteins or polypeptides comprising or essentially consisting of one or more of such parts, fragments, analogs, mutants, variants, alleles and/or derivatives, as long as these are suitable for the uses envisaged herein. Such parts, fragments, analogs, mutants, variants, alleles and/or derivatives will usually contain (at least part of) a functional antigen-binding site for binding against Dkk-1; and more preferably will be capable of specific binding to Dkk-1, and even more preferably capable of binding to Dkk-1 with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein. Some non-limiting examples of such parts, fragments, analogs, mutants, variants, alleles, derivatives, proteins and/or polypeptides will become clear from the further description herein. Additional fragments or polypeptides of the invention may also be provided by suitably combining (i.e. by linking or genetic fusion) one or more (smaller) parts or fragments as described herein.

In one specific, but non-limiting aspect of the invention, which will be further described herein, such analogs, mutants, variants, alleles, derivatives have an increased half-life in serum (as further described herein) compared to the amino acid sequence from which they have been derived. For example, an amino acid sequence of the invention may be linked (chemically or otherwise) to one or more groups or moieties that extend the half-life (such as PEG), so as to provide a derivative of an amino acid sequence of the invention with increased half-life.

In one specific, but non-limiting aspect, the amino acid sequence of the invention may be an amino acid sequence that comprises an immunoglobulin fold or may be an amino acid sequence that, under suitable conditions (such as physiological conditions) is capable of forming an immunoglobulin fold (i.e. by folding). Reference is inter alia made to the review by Halaby et al. (J. (1999) Protein Eng. 12, 563-71). Preferably, when properly folded so as to form an immunoglobulin fold, such an amino acid sequence is capable of specific binding (as defined herein) to Dkk-1; and more preferably capable of binding to Dkk-1 with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein. Also, parts, fragments, analogs, mutants, variants, alleles and/or derivatives of such amino acid sequences are preferably such that they comprise an immunoglobulin fold or are capable for forming, under suitable conditions, an immunoglobulin fold.

In particular, but without limitation, the amino acid sequences of the invention may be amino acid sequences that essentially consist of 4 framework regions (FR1 to FR4 respectively) and 3

complementarity determining regions (CDR1 to CDR3 respectively); or any suitable fragment of such an amino acid sequence (which will then usually contain at least some of the amino acid residues that form at least one of the CDR's, as further described herein).

The amino acid sequences of the invention may in particular be an immunoglobulin sequence or a suitable fragment thereof, and more in particular be an immunoglobulin variable domain sequence or a suitable fragment thereof, such as light chain variable domain sequence (e.g. a V_L-sequence) or a suitable fragment thereof; or a heavy chain variable domain sequence (e.g. a V_H-sequence) or a suitable fragment thereof. When the amino acid sequence of the invention is a heavy chain variable domain sequence, it may be a heavy chain variable domain sequence that is derived from a conventional four-chain antibody (such as, without limitation, a V_H sequence that is derived from a human antibody) or be a so-called V_{HH}-sequence (as defined herein) that is derived from a so-called "heavy chain antibody" (as defined herein).

However, it should be noted that the invention is not limited as to the origin of the amino acid sequence of the invention (or of the nucleotide sequence of the invention used to express it), nor as to the way that the amino acid sequence or nucleotide sequence of the invention is (or has been) generated or obtained. Thus, the amino acid sequences of the invention may be naturally occurring amino acid sequences (from any suitable species) or synthetic or semi-synthetic amino acid sequences. In a specific but non-limiting aspect of the invention, the amino acid sequence is a naturally occurring immunoglobulin sequence (from any suitable species) or a synthetic or semi-synthetic immunoglobulin sequence, including but not limited to "humanized" (as defined herein) immunoglobulin sequences (such as partially or fully humanized mouse or rabbit immunoglobulin sequences, and in particular partially or fully humanized V_{HH} sequences or Nanobodies®), "camelized" (as defined herein) immunoglobulin sequences, as well as immunoglobulin sequences that have been obtained by techniques such as affinity maturation (for example, starting from synthetic, random or naturally occurring immunoglobulin sequences), CDR grafting, veneering, combining fragments derived from different immunoglobulin sequences, PCR assembly using overlapping primers, and similar techniques for engineering immunoglobulin sequences well known to the skilled person; or any suitable combination of any of the foregoing. Reference is for example made to the standard handbooks, as well as to the further description and prior art mentioned herein.

Similarly, the nucleotide sequences of the invention may be naturally occurring nucleotide sequences or synthetic or semi-synthetic sequences, and may for example be sequences that are isolated by PCR from a suitable naturally occurring template (e.g. DNA or RNA isolated from a cell), nucleotide sequences that have been isolated from a library (and in particular, an expression library),

nucleotide sequences that have been prepared by introducing mutations into a naturally occurring nucleotide sequence (using any suitable technique known per se, such as mismatch PCR), nucleotide sequence that have been prepared by PCR using overlapping primers, or nucleotide sequences that have been prepared using techniques for DNA synthesis known per se.

The amino acid sequence of the invention may in particular be a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody), a single domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody), a "dAb" (or an amino acid sequence that is suitable for use as a dAb) or a Nanobody®™ (as defined herein, and including but not limited to a V_{HH} sequence); other single variable domains, or any suitable fragment of any one thereof. For a general description of (single) domain antibodies, reference is also made to the prior art cited above, as well as to EP 0 368 684. For the term "dAb's", reference is for example made to Ward et al. (1989, Nature 341 (6242): 544-6), to Holt et al., Trends Biotechnol., 2003, 21(11):484-490; as well as to for example WO 06/030220, WO 06/003388 and other published patent applications of Domantis Ltd. It should also be noted that, although less preferred in the context of the present invention because they are not of mammalian origin, single domain antibodies or single variable domains can be derived from certain species of shark (for example, the so-called "IgNAR domains", see for example WO 05/18629).

In particular, the amino acid sequence of the invention may be a Nanobody® (as defined herein) or a suitable fragment thereof. *[Note: Nanobody®, Nanobodies® and Nanoclone® are registered trademarks of Ablynx N.V.]* Such Nanobodies® directed against Dkk-1 will also be referred to herein as "*Nanobodies® of the invention*".

For a general description of Nanobodies®, reference is made to the further description below, as well as to the prior art cited herein. In this respect, it should however be noted that this description and the prior art mainly described Nanobodies® of the so-called "V_H3 class" (i.e. Nanobodies® with a high degree of sequence homology to human germline sequences of the V_H3 class such as DP-47, DP-51 or DP-29), which Nanobodies® form a preferred aspect of this invention. It should however be noted that the invention in its broadest sense generally covers any type of Nanobody® directed against Dkk-1, and for example also covers the Nanobodies® belonging to the so-called "V_H4 class" (i.e. Nanobodies® with a high degree of sequence homology to human germline sequences of the V_H4 class such as DP-78), as for example described in WO 07/118670.

Generally, Nanobodies® (in particular V_{HH} sequences and partially humanized Nanobodies®) can in particular be characterized by the presence of one or more "*Hallmark residues*" (as described herein) in one or more of the framework sequences (again as further described herein).

Thus, generally, a Nanobody® can be defined as an amino acid sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which one or more of the Hallmark residues are as further defined herein.

In particular, a Nanobody® can be an amino acid sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which the framework sequences are as further defined herein.

More in particular, a Nanobody® can be an amino acid sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2 below;

and in which:

ii) said amino acid sequence has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1 to 22, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences (indicated with X in the sequences of SEQ ID NO's: 1 to 22) are disregarded.

In these Nanobodies®, the CDR sequences are generally as further defined herein.

Thus, the invention also relates to such Nanobodies® that can bind to (as defined herein) and/or are directed against Dkk-1, to suitable fragments thereof, as well as to polypeptides that comprise or essentially consist of one or more of such Nanobodies® and/or suitable fragments.

SEQ ID NO's: 1589-1797 (see Table A-1) give the amino acid sequences of a number of V_{HH} sequences that have been raised against Dkk-1.

In particular, the invention in some specific aspects provides:

- amino acid sequences that are directed against (as defined herein) Dkk-1 and that have at least 80%, preferably at least 85%, such as 90% or 95% or more sequence identity with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1). These amino acid sequences may further be such that they neutralize binding of LRP5/6 or Kremen to Dkk-1; and/or compete with LRP5/6 or Kremen for binding to Dkk-1; and/or are directed against an interaction site (as defined herein) on Dkk-1 (such as the LRP5/6 or Kremen binding site);
- amino acid sequences that cross-block (as defined herein) the binding of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1) to Dkk-1 and/or that compete with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1) for binding to Dkk-1. Again, these amino acid sequences may further be such that they neutralize binding of the LRP5/6 or Kremen to Dkk-1; and/or compete with LRP5/6 or Kremen for binding to Dkk-1; and/or are directed against an interaction site (as defined herein) on Dkk-1 (such as the LRP5/6 or Kremen binding site);

which amino acid sequences may be as further described herein (and may for example be Nanobodies®); as well as polypeptides of the invention that comprise one or more of such amino acid sequences (which may be as further described herein, and may for example be bispecific and/or biparatopic polypeptides as described herein), and nucleic acid sequences that encode such amino acid sequences and polypeptides. Such amino acid sequences and polypeptides do not include any naturally occurring ligands.

In some other specific aspects, the invention provides:

- amino acid sequences of the invention that are specific for Dkk-1 compared to Dkk-2, Dkk-3 and/or Dkk-4;
- amino acid sequences of the invention that are specific for Dkk-1, Dkk-2 and Dkk-4 compared to Dkk-3;
- amino acid sequences of the invention that are specific for Dkk-1 and Dkk-4 compared to Dkk-2 and/or Dkk-3

Accordingly, the amino acid sequences and polypeptides of the invention may or may not be cross reactivity with different Dkk family members. In one aspect, the amino acid sequences and polypeptides of the invention are reactive with Dkk-1 while they are not reactive with Dkk-2, Dkk-3 or Dkk-4. In another aspect, the amino acid sequences and polypeptides of the invention are reactive with Dkk-1, Dkk-2 and Dkk-4 while they are not reactive with Dkk-3. In another aspect, the amino

acid sequences and polypeptides of the invention are reactive with Dkk-1 and Dkk-4 while they are not reactive with Dkk-2 or Dkk-3; which amino acid sequences of the invention may be as further described herein (and may for example be Nanobodies®); as well as polypeptides of the invention that comprise one or more of such amino acid sequences (which may be as further described herein, and may for example be bispecific and/or biparatopic polypeptides as described herein), and nucleic acid sequences that encode such amino acid sequences and polypeptides. Such amino acid sequences and polypeptides do not include any naturally occurring ligands.

Accordingly, some particularly preferred Nanobodies® of the invention are Nanobodies® which can bind (as further defined herein) to and/or are directed against to Dkk-1 and which:

i) have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1), in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded. In this respect, reference is also made to Table B-1, which lists the framework 1 sequences (SEQ ID NO's: 126-334), framework 2 sequences (SEQ ID NO's: 544-752), framework 3 sequences (SEQ ID NO's: 962-1170) and framework 4 sequences (SEQ ID NO's: 1380-1588) of the Nanobodies® of SEQ ID NO's: 1589-1797 (see Table A-1) (with respect to the amino acid residues at positions 1 to 4 and 27 to 30 of the framework 1 sequences, reference is also made to the comments made below. Thus, for determining the degree of amino acid identity, these residues are preferably disregarded);

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2 below.

In these Nanobodies®, the CDR sequences are generally as further defined herein.

Again, such Nanobodies® may be derived in any suitable manner and from any suitable source, and may for example be naturally occurring V_{HH} sequences (i.e. from a suitable species of Camelid) or synthetic or semi-synthetic amino acid sequences, including but not limited to "humanized" (as defined herein) Nanobodies®, "camelized" (as defined herein) immunoglobulin sequences (and in particular camelized heavy chain variable domain sequences), as well as Nanobodies® that have been obtained by techniques such as affinity maturation (for example, starting from synthetic, random or naturally occurring immunoglobulin sequences), CDR grafting, veneering, combining fragments derived from different immunoglobulin sequences, PCR assembly using overlapping primers, and similar techniques for engineering immunoglobulin sequences well known to the skilled person; or any suitable combination of any of the foregoing as further described

herein. Also, when a Nanobody® comprises a V_{HH} sequence, said Nanobody® may be suitably humanized, as further described herein, so as to provide one or more further (partially or fully) humanized Nanobodies® of the invention. Similarly, when a Nanobody® comprises a synthetic or semi-synthetic sequence (such as a partially humanized sequence), said Nanobody® may optionally be further suitably humanized, again as described herein, again so as to provide one or more further (partially or fully) humanized Nanobodies® of the invention.

In particular, humanized Nanobodies® may be amino acid sequences that are as generally defined for Nanobodies® in the previous paragraphs, but in which at least one amino acid residue is present (and in particular, in at least one of the framework residues) that is and/or that corresponds to a humanizing substitution (as defined herein). Some preferred, but non-limiting humanizing substitutions (and suitable combinations thereof) will become clear to the skilled person based on the disclosure herein. In addition, or alternatively, other potentially useful humanizing substitutions can be ascertained by comparing the sequence of the framework regions of a naturally occurring V_{HH} sequence with the corresponding framework sequence of one or more closely related human V_H sequences, after which one or more of the potentially useful humanizing substitutions (or combinations thereof) thus determined can be introduced into said V_{HH} sequence (in any manner known per se, as further described herein) and the resulting humanized V_{HH} sequences can be tested for affinity for the target, for stability, for ease and level of expression, and/or for other desired properties. In this way, by means of a limited degree of trial and error, other suitable humanizing substitutions (or suitable combinations thereof) can be determined by the skilled person based on the disclosure herein. Also, based on the foregoing, (the framework regions of) a Nanobody® may be partially humanized or fully humanized.

Some particularly preferred humanized Nanobodies® of the invention are humanized variants of the Nanobodies® of SEQ ID NO's: 1589-1797 (see Table A-1), of which the amino acid sequences of SEQ ID NO's: 1798-1816 (see Table A-2) are some especially preferred examples.

Thus, some other preferred Nanobodies® of the invention are Nanobodies® which can bind (as further defined herein) to Dkk-1 and which:

i) are a humanized variant of one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1); and/or

ii) have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1) and/or at least one of the amino acid sequences of SEQ ID NO's: 1798-1816 (see Table A-2), in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

i) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2 below.

According to another specific aspect of the invention, the invention provides a number of stretches of amino acid residues (i.e. small peptides) that are particularly suited for binding to Dkk-1. These stretches of amino acid residues may be present in, and/or may be incorporated into, an amino acid sequence of the invention, in particular in such a way that they form (part of) the antigen binding site of an amino acid sequence of the invention. As these stretches of amino acid residues were first generated as CDR sequences of heavy chain antibodies or V_{HH} sequences that were raised against Dkk-1 (or may be based on and/or derived from such CDR sequences, as further described herein), they will also generally be referred to herein as "*CDR sequences*" (i.e. as CDR1 sequences, CDR2 sequences and CDR3 sequences, respectively). It should however be noted that the invention in its broadest sense is not limited to a specific structural role or function that these stretches of amino acid residues may have in an amino acid sequence of the invention, as long as these stretches of amino acid residues allow the amino acid sequence of the invention to bind to Dkk-1. Thus, generally, the invention in its broadest sense comprises any amino acid sequence that is capable of binding to Dkk-1 and that comprises one or more CDR sequences as described herein, and in particular a suitable combination of two or more such CDR sequences, that are suitably linked to each other via one or more further amino acid sequences, such that the entire amino acid sequence forms a binding domain and/or binding unit that is capable of binding to Dkk-1. It should however also be noted that the presence of only one such CDR sequence in an amino acid sequence of the invention may by itself already be sufficient to provide an amino acid sequence of the invention that is capable of binding to Dkk-1; reference is for example again made to the so-called "Expedite fragments" described in WO 03/050531.

Thus, in another specific, but non-limiting aspect, the amino acid sequence of the invention may be an amino acid sequence that comprises at least one amino acid sequence that is chosen from the group consisting of the CDR1 sequences, CDR2 sequences and CDR3 sequences that are described herein (or any suitable combination thereof). In particular, an amino acid sequence of the invention may be an amino acid sequence that comprises at least one antigen binding site, wherein said antigen binding site comprises at least one amino acid sequence that is chosen from the group consisting of the CDR1 sequences, CDR2 sequences and CDR3 sequences that are described herein (or any suitable combination thereof).

Generally, in this aspect of the invention, the amino acid sequence of the invention may be any amino acid sequence that comprises at least one stretch of amino acid residues, in which said

stretch of amino acid residues has an amino acid sequence that corresponds to the sequence of at least one of the CDR sequences described herein. Such an amino acid sequence may or may not comprise an immunoglobulin fold. For example, and without limitation, such an amino acid sequence may be a suitable fragment of an immunoglobulin sequence that comprises at least one such CDR sequence, but that is not large enough to form a (complete) immunoglobulin fold (reference is for example again made to the "Expedite fragments" described in WO 03/050531). Alternatively, such an amino acid sequence may be a suitable "protein scaffold" that comprises least one stretch of amino acid residues that corresponds to such a CDR sequence (i.e. as part of its antigen binding site). Suitable scaffolds for presenting amino acid sequences will be clear to the skilled person, and for example comprise, without limitation, to binding scaffolds based on or derived from immunoglobulins (i.e. other than the immunoglobulin sequences already described herein), protein scaffolds derived from protein A domains (such as Affibodies™), tendamistat, fibronectin, lipocalin, CTLA-4, T-cell receptors, designed ankyrin repeats, avimers and PDZ domains (Binz et al., Nat. Biotech 2005, Vol 23:1257), and binding moieties based on DNA or RNA including but not limited to DNA or RNA aptamers (Ulrich et al., Comb Chem High Throughput Screen 2006 9(8):619-32).

Again, any amino acid sequence of the invention that comprises one or more of these CDR sequences is preferably such that it can specifically bind (as defined herein) to Dkk-1, and more in particular such that it can bind to Dkk-1 with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50} value, as further described herein), that is as defined herein.

More in particular, the amino acid sequences according to this aspect of the invention may be any amino acid sequence that comprises at least one antigen binding site, wherein said antigen binding site comprises at least two amino acid sequences that are chosen from the group consisting of the CDR1 sequences described herein, the CDR2 sequences described herein and the CDR3 sequences described herein, such that (i) when the first amino acid sequence is chosen from the CDR1 sequences described herein, the second amino acid sequence is chosen from the CDR2 sequences described herein or the CDR3 sequences described herein; (ii) when the first amino acid sequence is chosen from the CDR2 sequences described herein, the second amino acid sequence is chosen from the CDR1 sequences described herein or the CDR3 sequences described herein; or (iii) when the first amino acid sequence is chosen from the CDR3 sequences described herein, the second amino acid sequence is chosen from the CDR1 sequences described herein or the CDR3 sequences described herein.

Even more in particular, the amino acid sequences of the invention may be amino acid sequences that comprise at least one antigen binding site, wherein said antigen binding site comprises at least three amino acid sequences that are chosen from the group consisting of the CDR1 sequences described herein, the CDR2 sequences described herein and the CDR3 sequences described herein, such that the first amino acid sequence is chosen from the CDR1 sequences described herein, the second amino acid sequence is chosen from the CDR2 sequences described herein, and the third amino acid sequence is chosen from the CDR3 sequences described herein. Preferred combinations of CDR1, CDR2 and CDR3 sequences will become clear from the further description herein. As will be clear to the skilled person, such an amino acid sequence is preferably an immunoglobulin sequence (as further described herein), but it may for example also be any other amino acid sequence that comprises a suitable scaffold for presenting said CDR sequences.

Thus, in one specific, but non-limiting aspect, the invention relates to an amino acid sequence directed against Dkk-1, that comprises one or more stretches of amino acid residues chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 355-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 355-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 355-543;

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

or any suitable combination thereof.

When an amino acid sequence of the invention contains one or more amino acid sequences according to b) and/or c):

i) any amino acid substitution in such an amino acid sequence according to b) and/or c) is preferably, and compared to the corresponding amino acid sequence according to a), a conservative amino acid substitution, (as defined herein);

and/or

ii) the amino acid sequence according to b) and/or c) preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding amino acid sequence according to a);

and/or

iii) the amino acid sequence according to b) and/or c) may be an amino acid sequence that is derived from an amino acid sequence according to a) by means of affinity maturation using one or more techniques of affinity maturation known per se.

Similarly, when an amino acid sequence of the invention contains one or more amino acid sequences according to e) and/or f):

i) any amino acid substitution in such an amino acid sequence according to e) and/or f) is preferably, and compared to the corresponding amino acid sequence according to d), a conservative amino acid substitution, (as defined herein);

and/or

ii) the amino acid sequence according to e) and/or f) preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding amino acid sequence according to d);

and/or

iii) the amino acid sequence according to e) and/or f) may be an amino acid sequence that is derived from an amino acid sequence according to d) by means of affinity maturation using one or more techniques of affinity maturation known per se.

Also, similarly, when an amino acid sequence of the invention contains one or more amino acid sequences according to h) and/or i):

i) any amino acid substitution in such an amino acid sequence according to h) and/or i) is preferably, and compared to the corresponding amino acid sequence according to g), a conservative amino acid substitution, (as defined herein);

and/or

ii) the amino acid sequence according to h) and/or i) preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding amino acid sequence according to g);

and/or

iii) the amino acid sequence according to h) and/or i) may be an amino acid sequence that is derived from an amino acid sequence according to g) by means of affinity maturation using one or more techniques of affinity maturation known per se.

It should be understood that the last preceding paragraphs also generally apply to any amino acid sequences of the invention that comprise one or more amino acid sequences according to b), c), e), f), h) or i), respectively.

In this specific aspect, the amino acid sequence preferably comprises one or more stretches of amino acid residues chosen from the group consisting of:

i) the amino acid sequences of SEQ ID NO's: 355-543;

ii) the amino acid sequences of SEQ ID NO's: 753-961; and

iii) the amino acid sequences of SEQ ID NO's: 1171-1379;

or any suitable combination thereof.

Also, preferably, in such an amino acid sequence, the at least one of said stretches of amino acid residues forms part of the antigen binding site for binding against Dkk-1.

In a more specific, but again non-limiting aspect, the invention relates to an amino acid sequence directed against Dkk-1, that comprises two or more stretches of amino acid residues chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 355-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 355-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 355-543;

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

such that (i) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to a), b) or c), the second stretch of amino acid residues corresponds to one of

the amino acid sequences according to d), e), f), g), h) or i); (ii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to d), e) or f), the second stretch of amino acid residues corresponds to one of the amino acid sequences according to a), b), c), g), h) or i); or (iii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to g), h) or i), the second stretch of amino acid residues corresponds to one of the amino acid sequences according to a), b), c), d), e) or f).

In this specific aspect, the amino acid sequence preferably comprises two or more stretches of amino acid residues chosen from the group consisting of:

i) the amino acid sequences of SEQ ID NO's: 355-543;

ii) the amino acid sequences of SEQ ID NO's: 753-961; and

iii) the amino acid sequences of SEQ ID NO's: 1171-1379;

such that, (i) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 355-543, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 753-961 or of SEQ ID NO's: 1171-1379; (ii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 753-961, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 355-543 or of SEQ ID NO's: 1171-1379; or (iii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 1171-1379, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 355-543 or of SEQ ID NO's: 753-961.

Also, in such an amino acid sequence, the at least two stretches of amino acid residues again preferably form part of the antigen binding site for binding against Dkk-1.

In an even more specific, but non-limiting aspect, the invention relates to an amino acid sequence directed against Dkk-1, that comprises three or more stretches of amino acid residues, in which the first stretch of amino acid residues is chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 355-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 355-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 355-543;

the second stretch of amino acid residues is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

and the third stretch of amino acid residues is chosen from the group consisting of:

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379.

Preferably, in this specific aspect, the first stretch of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 355-543; the second stretch of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 753-961; and the third stretch of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 1171-1379.

Again, preferably, in such an amino acid sequence, the at least three stretches of amino acid residues forms part of the antigen binding site for binding against Dkk-1.

Preferred combinations of such stretches of amino acid sequences will become clear from the further disclosure herein.

Preferably, in such amino acid sequences the CDR sequences have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1). This degree of amino acid identity can for example be determined by determining the degree of amino acid identity (in a manner described herein) between said amino acid sequence and one or more of the sequences of SEQ ID NO's: 1589-1797 (see Table A-1), in which the amino acid residues that form the framework regions are disregarded. Also, such amino acid sequences of the invention can be as further described herein.

Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to Dkk-1; and more in particular bind to Dkk-1 with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein.

When the amino acid sequence of the invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), the amino acid sequence of the invention is preferably such that:

- CDR1 is chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 335-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

and/or

- CDR2 is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

and/or

- CDR3 is chosen from the group consisting of:

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379.

In particular, such an amino acid sequence of the invention may be such that CDR1 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 335-543; and/or CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 753-961; and/or CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 1171-1379.

In particular, when the amino acid sequence of the invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), the amino acid sequence of the invention is preferably such that:

- CDR1 is chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 335-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

and

- CDR2 is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

and

- CDR3 is chosen from the group consisting of:

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379; or any suitable fragment of such an amino acid sequence

In particular, such an amino acid sequence of the invention may be such that CDR1 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 335-543; and CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 753-961; and CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 1171-1379].

Again, preferred combinations of CDR sequences will become clear from the further description herein.

Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to Dkk-1; and more in particular bind to Dkk-1 with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein.

In one preferred, but non-limiting aspect, the invention relates to an amino acid sequence that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which the CDR sequences of said amino acid sequence have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1). This degree of amino acid identity can for example be determined by determining the degree of amino acid identity (in a manner described herein) between said amino acid sequence and one or more of the sequences of SEQ ID NO's: 1589-

1797 (see Table A-1), in which the amino acid residues that form the framework regions are disregarded. Such amino acid sequences of the invention can be as further described herein.

In such an amino acid sequence of the invention, the framework sequences may be any suitable framework sequences, and examples of suitable framework sequences will be clear to the skilled person, for example on the basis the standard handbooks and the further disclosure and prior art mentioned herein.

The framework sequences are preferably (a suitable combination of) immunoglobulin framework sequences or framework sequences that have been derived from immunoglobulin framework sequences (for example, by humanization or camelization). For example, the framework sequences may be framework sequences derived from a light chain variable domain (e.g. a V_L-sequence) and/or from a heavy chain variable domain (e.g. a V_H-sequence). In one particularly preferred aspect, the framework sequences are either framework sequences that have been derived from a V_{HH}-sequence (in which said framework sequences may optionally have been partially or fully humanized) or are conventional V_H sequences that have been camelized (as defined herein).

The framework sequences are preferably such that the amino acid sequence of the invention is a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody); is a single domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody); is a "dAb" (or an amino acid sequence that is suitable for use as a dAb); or is a Nanobody® (including but not limited to V_{HH} sequence). Again, suitable framework sequences will be clear to the skilled person, for example on the basis the standard handbooks and the further disclosure and prior art mentioned herein.

In particular, the framework sequences present in the amino acid sequences of the invention may contain one or more of Hallmark residues (as defined herein), such that the amino acid sequence of the invention is a Nanobody®. Some preferred, but non-limiting examples of (suitable combinations of) such framework sequences will become clear from the further disclosure herein.

Again, as generally described herein for the amino acid sequences of the invention, it is also possible to use suitable fragments (or combinations of fragments) of any of the foregoing, such as fragments that contain one or more CDR sequences, suitably flanked by and/or linked via one or more framework sequences (for example, in the same order as these CDR's and framework sequences may occur in the full-sized immunoglobulin sequence from which the fragment has been derived). Such fragments may also again be such that they comprise or can form an immunoglobulin fold, or alternatively be such that they do not comprise or cannot form an immunoglobulin fold.

In one specific aspect, such a fragment comprises a single CDR sequence as described herein (and in particular a CDR3 sequence), that is flanked on each side by (part of) a framework sequence

(and in particular, part of the framework sequence(s) that, in the immunoglobulin sequence from which the fragment is derived, are adjacent to said CDR sequence. For example, a CDR3 sequence may be preceded by (part of) a FR3 sequence and followed by (part of) a FR4 sequence). Such a fragment may also contain a disulphide bridge, and in particular a disulphide bridge that links the two framework regions that precede and follow the CDR sequence, respectively (for the purpose of forming such a disulphide bridge, cysteine residues that naturally occur in said framework regions may be used, or alternatively cysteine residues may be synthetically added to or introduced into said framework regions). For a further description of these "Expedite fragments", reference is again made to WO 03/050531, as well as to the US provisional application of Ablynx N.V. entitled *"Peptides capable of binding to serum proteins"* of Ablynx N.V. (inventors: Revets, Hilde Adi Pierrette; Kolkman, Joost Alexander; and Hoogenboom, Hendricus Renerus Jacobus Mattheus) filed on December 5, 2006 (see also PCT/EP2007/063348).

In another aspect, the invention relates to a compound or construct, and in particular a protein or polypeptide (also referred to herein as a *"compound of the invention"* or *"polypeptide of the invention"*, respectively) that comprises or essentially consists of one or more amino acid sequences of the invention (or suitable fragments thereof), and optionally further comprises one or more other groups, residues, moieties or binding units. As will become clear to the skilled person from the further disclosure herein, such further groups, residues, moieties, binding units or amino acid sequences may or may not provide further functionality to the amino acid sequence of the invention (and/or to the compound or construct in which it is present) and may or may not modify the properties of the amino acid sequence of the invention.

For example, such further groups, residues, moieties or binding units may be one or more additional amino acid sequences, such that the compound or construct is a (fusion) protein or (fusion) polypeptide. In a preferred but non-limiting aspect, said one or more other groups, residues, moieties or binding units are immunoglobulin sequences. Even more preferably, said one or more other groups, residues, moieties or binding units are chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s, amino acid sequences that are suitable for use as a dAb, or Nanobodies®.

Alternatively, such groups, residues, moieties or binding units may for example be chemical groups, residues, moieties, which may or may not by themselves be biologically and/or pharmacologically active. For example, and without limitation, such groups may be linked to the one or more amino acid sequences of the invention so as to provide a "derivative" of an amino acid sequence or polypeptide of the invention, as further described herein.

Also within the scope of the present invention are compounds or constructs, that comprises or essentially consists of one or more derivatives as described herein, and optionally further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers. Preferably, said one or more other groups, residues, moieties or binding units are amino acid sequences.

In the compounds or constructs described above, the one or more amino acid sequences of the invention and the one or more groups, residues, moieties or binding units may be linked directly to each other and/or via one or more suitable linkers or spacers. For example, when the one or more groups, residues, moieties or binding units are amino acid sequences, the linkers may also be amino acid sequences, so that the resulting compound or construct is a fusion (protein) or fusion (polypeptide).

As will be clear from the further description above and herein, this means that the amino acid sequences of the invention can be used as "building blocks" to form polypeptides of the invention, i.e. by suitably combining them with other groups, residues, moieties or binding units, in order to form compounds or constructs as described herein (such as, without limitations, the biparatopic. bi/multivalent and bi/multispecific polypeptides of the invention described herein) which combine within one molecule one or more desired properties or biological functions.

The compounds or polypeptides of the invention can generally be prepared by a method which comprises at least one step of suitably linking the one or more amino acid sequences of the invention to the one or more further groups, residues, moieties or binding units, optionally via the one or more suitable linkers, so as to provide the compound or polypeptide of the invention. Polypeptides of the invention can also be prepared by a method which generally comprises at least the steps of providing a nucleic acid that encodes a polypeptide of the invention, expressing said nucleic acid in a suitable manner, and recovering the expressed polypeptide of the invention. Such methods can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the methods and techniques further described herein.

The process of designing/selecting and/or preparing a compound or polypeptide of the invention, starting from an amino acid sequence of the invention, is also referred to herein as "*formatting*" said amino acid sequence of the invention; and an amino acid of the invention that is made part of a compound or polypeptide of the invention is said to be "*formatted*" or to be "*in the format of*" said compound or polypeptide of the invention. Examples of ways in which an amino acid sequence of the invention can be formatted and examples of such formats will be clear to the skilled person based on the disclosure herein; and such formatted amino acid sequences form a further aspect of the invention.

In one specific aspect of the invention, a compound of the invention or a polypeptide of the invention may have an increased half-life, compared to the corresponding amino acid sequence of the invention. Some preferred, but non-limiting examples of such compounds and polypeptides will become clear to the skilled person based on the further disclosure herein, and for example comprise amino acid sequences or polypeptides of the invention that have been chemically modified to increase the half-life thereof (for example, by means of pegylation); amino acid sequences of the invention that comprise at least one additional binding site for binding to a serum protein (such as serum albumin); or polypeptides of the invention that comprise at least one amino acid sequence of the invention that is linked to at least one moiety (and in particular at least one amino acid sequence) that increases the half-life of the amino acid sequence of the invention. Examples of polypeptides of the invention that comprise such half-life extending moieties or amino acid sequences will become clear to the skilled person based on the further disclosure herein; and for example include, without limitation, polypeptides in which the one or more amino acid sequences of the invention are suitable linked to one or more serum proteins or fragments thereof (such as (human) serum albumin or suitable fragments thereof) or to one or more binding units that can bind to serum proteins (such as, for example, domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s, amino acid sequences that are suitable for use as a dAb, or Nanobodies® that can bind to serum proteins such as serum albumin (such as human serum albumin), serum immunoglobulins such as IgG, or transferrine; reference is made to the further description and references mentioned herein); polypeptides in which an amino acid sequence of the invention is linked to an Fc portion (such as a human Fc) or a suitable part or fragment thereof; or polypeptides in which the one or more amino acid sequences of the invention are suitable linked to one or more small proteins or peptides that can bind to serum proteins (such as, without limitation, the proteins and peptides described in WO 91/01743, WO 01/45746, WO 02/076489 and to the US provisional application of Ablynx N.V. entitled *"Peptides capable of binding to serum proteins"* of Ablynx N.V. filed on December 5, 2006 (see also PCT/EP2007/063348).

Generally, the compounds or polypeptides of the invention with increased half-life preferably have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding amino acid sequence of the invention per se. For example, the compounds or polypeptides of the invention with increased half-life may have a half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than

12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence of the invention per se.

In a preferred, but non-limiting aspect of the invention, such compounds or polypeptides of the invention have a serum half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence of the invention per se.

In another preferred, but non-limiting aspect of the invention, such compounds or polypeptides of the invention exhibit a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more. For example, compounds or polypeptides of the invention may have a half-life of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

In another aspect, the invention relates to a nucleic acid that encodes an amino acid sequence of the invention or a polypeptide of the invention (or a suitable fragment thereof). Such a nucleic acid will also be referred to herein as a "*nucleic acid of the invention*" and may for example be in the form of a genetic construct, as further described herein.

In another aspect, the invention relates to a host or host cell that expresses (or that under suitable circumstances is capable of expressing) an amino acid sequence of the invention and/or a polypeptide of the invention; and/or that contains a nucleic acid of the invention. Some preferred but non-limiting examples of such hosts or host cells will become clear from the further description herein.

The invention further relates to a product or composition containing or comprising at least one amino acid sequence of the invention, at least one polypeptide of the invention (or a suitable fragment thereof) and/or at least one nucleic acid of the invention, and optionally one or more further components of such compositions known per se, i.e. depending on the intended use of the composition. Such a product or composition may for example be a pharmaceutical composition (as described herein), a veterinary composition or a product or composition for diagnostic use (as also described herein). Some preferred but non-limiting examples of such products or compositions will become clear from the further description herein.

The invention also relates to the use of an amino acid sequence, Nanobody® or polypeptide of the invention, or of a composition comprising the same, in (methods or compositions for) modulating Dkk-1, either in vitro (e.g. in an in vitro or cellular assay) or in vivo (e.g. in an a single cell

or in a multicellular organism, and in particular in a mammal, and more in particular in a human being, such as in a human being that is at risk of or suffers from a Dkk-1-associated diseases or disorders).

The invention also relates to methods for modulating Dkk-1, either in vitro (e.g. in an in vitro or cellular assay) or in vivo (e.g. in an a single cell or multicellular organism, and in particular in a mammal, and more in particular in a human being, such as in a human being that is at risk of or suffers from a Dkk-1-associated diseases or disorders), which method comprises at least the step of contacting Dkk-1 with at least one amino acid sequence, Nanobody® or polypeptide of the invention, or with a composition comprising the same, in a manner and in an amount suitable to modulate Dkk-1, with at least one amino acid sequence, Nanobody® or polypeptide of the invention.

The invention also relates to the use of an one amino acid sequence, Nanobody® or polypeptide of the invention in the preparation of a composition (such as, without limitation, a pharmaceutical composition or preparation as further described herein) for modulating Dkk-1, either in vitro (e.g. in an in vitro or cellular assay) or in vivo (e.g. in an a single cell or multicellular organism, and in particular in a mammal, and more in particular in a human being, such as in a human being that is at risk of or suffers from a Dkk-1-associated diseases or disorders).

In the context of the present invention, "modulating" or "to modulate" generally means either reducing or inhibiting the activity of, or alternatively increasing the activity of, Dkk-1, as measured using a suitable in vitro, cellular or in vivo assay (such as those mentioned herein). In particular, "modulating" or "to modulate" may mean either reducing or inhibiting the activity of, or alternatively increasing the activity of Dkk-1, as measured using a suitable in vitro, cellular or in vivo assay (such as those mentioned herein), by at least 1%, preferably at least 5%, such as at least 10% or at least 25%, for example by at least 50%, at least 60%, at least 70%, at least 80%, or 90% or more, compared to activity of Dkk-1 in the same assay under the same conditions but without the presence of the amino acid sequence, Nanobody® or polypeptide of the invention.

As will be clear to the skilled person, "modulating" may also involve effecting a change (which may either be an increase or a decrease) in affinity, avidity, specificity and/or selectivity of Dkk-1 for one or more of its targets, ligands or substrates (such as e.g. LRP5/6 and/or Kremen); and/or effecting a change (which may either be an increase or a decrease) in the sensitivity of Dkk-1 for one or more conditions in the medium or surroundings in which Dkk-1 is present (such as pH, ion strength, the presence of co-factors, etc.), compared to the same conditions but without the presence of the amino acid sequence, Nanobody® or polypeptide of the invention. As will be clear to the skilled person, this may again be determined in any suitable manner and/or using any suitable assay known per se, such as the assays described herein or in the prior art cited herein.

"Modulating" may also mean effecting a change (i.e. an activity as an agonist or as an antagonist, respectively) with respect to one or more biological or physiological mechanisms, effects, responses, functions, pathways or activities in which Dkk-1 (or in which its substrate(s), ligand(s) or pathway(s) are involved, such as its (inhibition of) signaling pathway or metabolic pathway and their associated biological or physiological effects) is involved. Again, as will be clear to the skilled person, such an action as an agonist or an antagonist may be determined in any suitable manner and/or using any suitable (in vitro and usually cellular or in assay) assay known per se, such as the assays described herein or in the prior art cited herein. In particular, an action as an agonist or antagonist may be such that (inhibition of) an intended biological or physiological activity is increased or decreased, respectively, by at least 1%, preferably at least 5%, such as at least 10% or at least 25%, for example by at least 50%, at least 60%, at least 70%, at least 80%, or 90% or more, compared to (inhibition of) the biological or physiological activity in the same assay under the same conditions but without the presence of the amino acid sequence, Nanobody® or polypeptide of the invention.

Modulating may for example involve reducing or inhibiting the binding of Dkk-1 to one of its substrates or ligands (such as e.g. LRP5/6 and/or Kremen) and/or competing with a natural ligand, substrate for binding to Dkk-1. Modulating may also involve activating Dkk-1 or the mechanism or pathway in which it is involved. Modulating may be reversible or irreversible, but for pharmaceutical and pharmacological purposes will usually be in a reversible manner.

Without being limiting, in one aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will inhibit and/or block binding of LRP5/6 to Dkk-1. The amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will preferably inhibit binding of LRP5/6 to Dkk-1 by at least 1%, preferably at least 5%, such as at least 10%, for example 25% or more or even 50% or more and up to 75% or even more than 90% or more, compared to binding of LRP5/6 to Dkk-1 in the absence of the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same. In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will inhibit and/or block binding of LRP5/6 to Dkk-1 equally or better than BHQ880. In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will inhibit and/or block binding of LRP5/6 to Dkk-1 equally or better than RH2-18. In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will inhibit and/or block binding of LRP5/6 to Dkk-1 equally or better than 11H10.

In another aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will inhibit and/or block binding of Kremen to Dkk-1. The

amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will preferably inhibit binding of Kremen to Dkk-1 by at least 1%, preferably at least 5%, such as at least 10%, for example 25% or more or even 50% or more and up to 75% or even more than 90% or more, compared to binding of Kremen to Dkk-1 in the absence of the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same.

In another aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will inhibit and/or block binding of LRP5/6 to Dkk-1 and inhibit and/or block binding of Kremen to Dkk-1, preferably essentially simultaneously. The amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will preferably inhibit binding of LRP5/6 to Dkk-1 by at least 1%, preferably at least 5%, such as at least 10%, for example 25% or more or even 50% or more and up to 75% or even more than 90% or more, compared to binding of LRP5/6 to Dkk-1 in the absence of the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same; and the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will preferably inhibit binding of Kremen to Dkk-1 by at least 1%, preferably at least 5%, such as at least 10%, for example 25% or more or even 50% or more and up to 75% or even more than 90% or more, compared to binding of Kremen to Dkk-1 in the absence of the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same.

In another aspect, which is for example preferred for use in the prevention and treatment of bone loss and/or osteolytic lesions, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same is an antagonist of Dkk-1 and/or an antagonist of the inhibitory function of Dkk-1 on Wnt signaling (i.e. of the signaling that is caused by binding of LRP5/6 to members of the Wnt pathway) and/or will neutralize the inhibitory function of Dkk-1 on Wnt signaling (i.e. by at least 1%, preferably at least 5%, such as at least 10%, for example 25% or more or even 50% or more and up to 75% or even more than 90% or more, compared to the inhibitory function of Dkk-1 on signaling without the presence of the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same (but in the presence of Dkk-1), as determined in a suitable assay, for example in a TCF/Lef luciferase assay as described in WO 06/015373, WO 07/084344, WO 08/097510 and van Bezooijen RL et al. 2007, J. Bone Miner. Res. 22: 19-28). In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will neutralize the inhibitory function of Dkk-1 on Wnt signaling equally or better than BHQ880. In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will neutralize the inhibitory function of Dkk-1 on Wnt signaling equally or better than RH2-18.

In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will neutralize the inhibitory function of Dkk-1 on Wnt signaling equally or better than 11H10.

In another aspect, which is also preferred for use in the prevention and treatment of bone loss and/or osteolytic lesions, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same is will neutralize the inhibitory function of Dkk-1 on osteoblastic cell differentiation. The amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will preferably neutralize the inhibitory function of Dkk-1 on osteoblastic cell differentiation by at least 1%, preferably at least 5%, such as at least 10%, for example 25% or more or even 50% or more and up to 75% or even more than 90% or more, compared to the inhibitory function of Dkk-1 on osteoblastic cell differentiation in the absence of the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same (but in the presence of Dkk-1), for example as measured in an osteoblastic cell differentiation assay of mesenchymal pluripotent cell line (e.g. C3H10T1/2) as described in WO 08/097510). In a preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will neutralize the inhibitory function of Dkk-1 on osteoblastic cell differentiation equally or better than BHQ880. In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will neutralize the inhibitory function of Dkk-1 on osteoblastic cell differentiation equally or better than RH2-18. In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will neutralize the inhibitory function of Dkk-1 on osteoblastic cell differentiation equally or better than 11H10.

In another aspect, which is also preferred for use in the prevention and treatment of bone loss and/or osteolytic lesions, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same is will increase in bone mass. The amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will preferably increase in bone mass by at least 1%, preferably at least 5%, such as at least 10%, for example 25% or more or even 50% or more and up to 75% or even more than 90% or more, compared to the bone mass in the absence of the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same (in an age-matched reference), for example as measured in an intact mice models in which BMD is measured as described in WO 06/015373, in oviarectomized mice, or in a synergeneic mouse model as described in WO 07/084344. In a preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will increase in bone mass equally or better than BHQ880. In another preferred aspect, the

amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will increase in bone mass equally or better than RH2-18. In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will increase in bone mass equally or better than 11H10.

In another aspect, which is also preferred for use in the prevention and treatment of bone loss and/or osteolytic lesions, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same is will reduce bone lesions. The amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will preferably reduce bone lesions by at least 1%, preferably at least 5%, such as at least 10%, for example 25% or more or even 50% or more and up to 75% or even more than 90% or more, compared to the bone lesions in the absence of the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same (in an age-matched reference), for example as measured in an intact mice models in which bone lesions are measured as described in WO 06/015373, in oviarectomized mice, or in a synergeneic mouse model as described in WO 07/084344. In a preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will reduce bone lesions equally or better than BHQ880. In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will reduce bone lesions equally or better than RH2-18. In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will reduce bone lesions equally or better than 11H10.

In another aspect, which is, for example, preferred for use in the treatment of cancers and/or myeloma, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same is will reduce the tumor burden. The amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will preferably reduce the tumor burden by at least 1%, preferably at least 5%, such as at least 10%, for example 25% or more or even 50% or more and up to 75% or even more than 90% or more, compared to the tumor burden in the absence of the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same (in an age-matched reference), for example as measured in a mice engrafted with multiple myeloma cells expressing various levels of Dkk-1 (Yaccoby et al. 2007, Blood 109: 2106), in an osteolytic prostate tumor model (WO 07/084344; Kim et al. 2003, Clin. Cancer Res. 9: 1200), in a SCID mice model of human colon cancer xenografts (Lozupone et al. 2004, Cancer Research 64: 378–385), in a SCID mice model for multiple myeloma (Cosette 1998, Blood, 91: 4727-4737), in a SCID-hu mice model (McCune, et al. 1988, Science 241: 1632-9; Colorado State University, Fort Collins, Colo.; Jackson Laboratory, Bar Harbor, Me., Charles

River Laboratories, Wilmington, Mass.), in a 5TGM1 mouse model (Dian et al. 2005, Mol. Cancer Ther. 4: 91). In a preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will reduce the tumor burden equally or better than BHQ880. In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will reduce the tumor burden equally or better than RH2-18. In another preferred aspect, the amino acid sequence, Nanobody® or polypeptide of the invention or the composition comprising the same will reduce the tumor burden equally or better than 11H10.

In a preferred, but non-limiting aspect, a suitable antagonistic biparatopic (or multiparatopic) polypeptide of the invention is used, and more preferably one of the preferred biparatopic (or multiparatopic) polypeptides of the invention, as further described herein.

Different modes of action may be mediated by each one of the binding units (as further defined herein) of the biparatopic amino acid sequence, Nanobody® or polypeptide of the invention, wherein each binding unit binds at a different binding site of Dkk-1. In a preferred aspect, the biparatopic amino acid sequence, Nanobody® or polypeptide of the invention combine the modes of action induced by the inhibition and/or blocking of the LRP5/6 interaction with Dkk-1 and the modes of action induced by the inhibition and/or blocking of the Kremen interaction with Dkk-1.

Accordingly, the present invention also relates to a biparatopic amino acid sequence, Nanobody® or polypeptide of the invention or a composition comprising the same that combines two different modes of action each mediated by one of the binding units of the biparatopic amino acid sequence, Nanobody® or polypeptide of the invention, wherein each binding unit binds at a different binding site of Dkk-1.

The present invention also relates to a triparatopic amino acid sequence, Nanobody® or polypeptide of the invention or a composition comprising the same that combines two or three different modes of action each mediated by one of the binding units of the triparatopic amino acid sequence, Nanobody® or polypeptide of the invention, wherein each binding unit binds at a different binding site of Dkk-1.

More generally, the present invention relates to a multiparatopic amino acid sequence, Nanobody® or polypeptide of the invention or a composition comprising the same that combines two or more different modes of action each mediated by one of the binding units of the multiparatopic amino acid sequence, Nanobody® or polypeptide of the invention, wherein each binding unit binds at a different binding site of Dkk-1.

The invention further relates to methods for preparing or generating the amino acid sequences, polypeptides, nucleic acids, host cells, products and compositions described herein.

Some preferred but non-limiting examples of such methods will become clear from the further description herein.

Generally, these methods may comprise the steps of:

a) providing a set, collection or library of amino acid sequences; and

b) screening said set, collection or library of amino acid sequences for amino acid sequences that can bind to and/or have affinity for Dkk-1;

and

c) isolating the amino acid sequence(s) that can bind to and/or have affinity for Dkk-1.

In such a method, the set, collection or library of amino acid sequences may be any suitable set, collection or library of amino acid sequences. For example, the set, collection or library of amino acid sequences may be a set, collection or library of immunoglobulin sequences (as described herein), such as a naïve set, collection or library of immunoglobulin sequences; a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

Also, in such a method, the set, collection or library of amino acid sequences may be a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of amino acid sequences may be a set, collection or library of domain antibodies or single domain antibodies, or may be a set, collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of amino acid sequences may be an immune set, collection or library of immunoglobulin sequences, for example derived from a mammal that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

In the above methods, the set, collection or library of amino acid sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

In the above step b), the set, collection or library may for example be screened for (nucleic acid sequences that encode) amino acid sequences that can bind to the LRP5/6 binding site on Dkk-1

(and in particular to at least one of the amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular to at least one of the amino acid residues R242 or H210 of Dkk-1) and/or that compete with LRP5/6 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (nucleic acid sequences that encode) amino acid sequences that can bind to the BHQ880 binding site on Dkk-1 and/or that compete with BHQ880 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (nucleic acid sequences that encode) amino acid sequences that can bind to the RH2-18 binding site on Dkk-1 and/or that compete with RH2-18 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (nucleic acid sequences that encode) amino acid sequences that can bind to the 11H10 binding site on Dkk-1 and/or that compete with 11H10 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (nucleic acid sequences that encode) amino acid sequences that can bind to the Kremen binding site on Dkk-1 (and in particular to at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular to at least one of the amino acid residues R197 or K232 of Dkk-1) and/or that compete with Kremen for binding to Dkk-1.

In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with LRP5/6, BHQ880, RH2-18, 11H10 or Kremen, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

In another aspect, the method for generating amino acid sequences comprises at least the steps of:

a) providing a collection or sample of cells expressing amino acid sequences;

b) screening said collection or sample of cells for cells that express an amino acid sequence that can bind to and/or have affinity for Dkk-1;

and

c) either (i) isolating said amino acid sequence; or (ii) isolating from said cell a nucleic acid sequence that encodes said amino acid sequence, followed by expressing said amino acid sequence.

For example, when the desired amino acid sequence is an immunoglobulin sequence, the collection or sample of cells may for example be a collection or sample of B-cells. Also, in this

method, the sample of cells may be derived from a mammal that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The above method may be performed in any suitable manner, as will be clear to the skilled person. Reference is for example made to EP 0 542 810, WO 05/19824, WO 04/051268 and WO 04/106377. The screening of step b) is preferably performed using a flow cytometry technique such as FACS. For this, reference is for example made to Lieby et al., Blood, Vol. 97, No. 12, 3820 (2001).

Again, in the above step b), the set, collection or library may for example be screened for (cells that express) amino acid sequences that can bind to the LRP5/6 binding site on Dkk-1 (and in particular to at least one of the amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular to at least one of the amino acid residues R242 or H210 of Dkk-1) and/or that compete with LRP5/6 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (cells that express) amino acid sequences that can bind to the BHQ880 binding site on Dkk-1 and/or that compete with BHQ880 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (cells that express) amino acid sequences that can bind to the RH2-18 binding site on Dkk-1 and/or that compete with RH2-18 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (cells that express) amino acid sequences that can bind to the 11H10 binding site on Dkk-1 and/or that compete with 11H10 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (cells that express) amino acid sequences that can bind to the Kremen binding site on Dkk-1 (and in particular to at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular to at least one of the amino acid residues R197 or K232 of Dkk-1) and/or that compete with Kremen for binding to Dkk-1.

In another aspect, the method for generating an amino acid sequence directed against Dkk-1 may comprise at least the steps of:

a) providing a set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1;

and

c) isolating said nucleic acid sequence, followed by expressing said amino acid sequence.

In such a method, the set, collection or library of nucleic acid sequences encoding amino acid sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

Also, in such a method, the set, collection or library of nucleic acid sequences may encode a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of nucleic acid sequences may encode a set, collection or library of domain antibodies or single domain antibodies, or a set, collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of nucleic acid sequences may be an immune set, collection or library of nucleic acid sequences, for example derived from a mammal that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The set, collection or library of nucleic acid sequences may for example encode an immune set, collection or library of heavy chain variable domains or of light chain variable domains. In one specific aspect, the set, collection or library of nucleotide sequences may encode a set, collection or library of V_{HH} sequences.

In the above methods, the set, collection or library of nucleotide sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) nucleotide sequences encoding amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

Again, in the above step b), the set, collection or library may for example be screened for (nucleic acid sequences that encode) amino acid sequences that can bind to the LRP5/6 binding site on Dkk-1 (and in particular to at least one of the amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular to at least one of the amino acid residues R242 or H210 of Dkk-1) and/or that compete with LRP5/6 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (nucleic acid sequences that encode) amino acid sequences that can bind to the BHQ880 binding site on Dkk-1 and/or that compete with BHQ880 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (nucleic acid sequences that encode) amino acid sequences that can bind to the RH2-18 binding site on Dkk-1 and/or that compete with RH2-18 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (nucleic acid sequences that encode) amino acid sequences that can bind to the 11H10 binding site on Dkk-1 and/or that compete with 11H10 for binding to Dkk-1.

Alternatively, in the above step b), the set, collection or library may for example be screened for (nucleic acid sequences that encode) amino acid sequences that can bind to the Kremen binding site on Dkk-1 (and in particular to at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular to at least one of the amino acid residues R197 or K232 of Dkk-1) and/or that compete with Kremen for binding to Dkk-1.

In another aspect, the method for generating an amino acid sequence directed against Dkk-1 may comprise at least the steps of:

a) providing a set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 and that is cross-blocked or is cross blocking a Nanobody® of the invention, e.g. one of SEQ ID NO: 1589-1797 (Table A-1), or a humanized Nanobody® of the invention, e.g. SEQ ID NO: 1798-1816 (see Table A-2), or a polypeptide or construct of the invention, e.g. SEQ ID NO: 1817-1820 (see Table A-3); and

c) isolating said nucleic acid sequence, followed by expressing said amino acid sequence.

Also encompassed within the present invention are methods for preparing and generating multiparatopic (such as e.g. biparatopic, triparatopic, etc.) amino acids of the invention.

Without being limiting, a method for preparing and generating biparatopic amino acids of the invention may comprise at least the steps of:

a) providing a nucleic acid sequence encoding a Dkk-1 binding amino acid sequence fused to a set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for an antigenic determinant on Dkk-1 different from the antigenic determinant recognized by the Dkk-1 binding amino acid sequence;

and

c) isolating the nucleic acid sequence encoding a Dkk-1 binding amino acid sequence fused to the nucleic acid sequence obtained in b), followed by expressing the encoded amino acid sequence.

The biparatopic amino acid sequence obtained in the method above, can subsequently be fused to one or more further sets, collections or libraries of nucleic acid sequences encoding amino acid sequences and again screened for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for an antigenic determinant on Dkk-1 different from the antigenic determinant on the Dkk-1 recognized by the Dkk-1 binding amino acid sequence and different from the antigenic determinant selected for in b) in order to obtain a triparatopic or multiparatopic amino acid sequence respectively.

In such a method, the set, collection or library of nucleic acid sequences encoding amino acid sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

Also, in such a method, the set, collection or library of nucleic acid sequences may encode a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of nucleic acid sequences may encode a set, collection or library of domain antibodies or single domain antibodies, or a set, collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of nucleic acid sequences may be an immune set, collection or library of nucleic acid sequences, for example derived from a mammal that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The set, collection or library of nucleic acid sequences may for example encode an immune set, collection or library of heavy chain variable domains or of light chain variable domains. In one specific aspect, the set, collection or library of nucleotide sequences may encode a set, collection or library of V_{HH} sequences.

In the above methods, the nucleic acid sequence encoding an Dkk-1 binding amino acid sequence fused to the set, collection or library of nucleotide sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) nucleotide sequences encoding amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

According to a particularly preferred aspect, a method for preparing and generating biparatopic amino acids of the invention may comprise at least the steps of:

a) providing a set, collection or library of nucleic acid sequences, in which each nucleic acid sequence in said set, collection or library encodes a fusion protein that comprises a first amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1 that is fused (optionally via a linker sequence) to a second amino acid sequence, in which essentially each second amino acid sequence (or most of these) is a different member of a set, collection or library of different amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on Dkk-1 different from the first antigenic determinant, part, domain or epitope on Dkk-1;

and

c) isolating the nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on Dkk-1 different from the first antigenic determinant, part, domain or epitope on Dkk-1, obtained in b), optionally followed by expressing the encoded amino acid sequence.

In this preferred method, the first amino acid sequence in the fusion protein encoded by said set collection or library of nucleic acid sequences may be the same amino acid sequence for all members of the set, collection or library of nucleic acid sequences encoding the fusion protein; or the first amino acid sequence in the fusion protein encoded by said set collection or library of nucleic acid sequences may also be a member of a set collection or library of different amino acid sequences.

Again, in such a method, the set, collection or library of nucleic acid sequences encoding amino acid sequences that form part of the fusion protein may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-

synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

Also, in such a method, the set, collection or library of nucleic acid sequences may encode a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of nucleic acid sequences may encode a set, collection or library of domain antibodies or single domain antibodies, or a set, collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of nucleic acid sequences may be an immune set, collection or library of nucleic acid sequences, for example derived from a mammal that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The set, collection or library of nucleic acid sequences may for example encode an immune set, collection or library of heavy chain variable domains or of light chain variable domains. In one specific aspect, the set, collection or library of nucleotide sequences may encode a set, collection or library of V_{HH} sequences.

In the above methods, the nucleic acid sequence encoding an Dkk-1 binding amino acid sequence fused to the set, collection or library of nucleotide sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) nucleotide sequences encoding amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

In step b), the set, collection or library of nucleic acid sequences may also be screened for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for both the first antigenic determinant, part, domain or epitope on Dkk-1 and the second antigenic determinant, part, domain or epitope on Dkk-1. This may for example be performed in a subsequent steps (i.e. by in a first step screening or selecting for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the second antigenic determinant, part, domain or epitope on Dkk-1, and subsequently in a second step selecting or screening for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the first

antigenic determinant, part, domain or epitope on Dkk-1; or visa versa) or in a single step (i.e. by simultaneously screening or selecting for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for both the first antigenic determinant, part, domain or epitope on Dkk-1 and the second antigenic determinant, part, domain or epitope on Dkk-1).

In a preferred aspect of the above method, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or (ii) it competes with LRP5/6 for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of the amino acid residues R197 or K232 of Dkk-1) and/or (ii) an amino acid sequence that can compete with Kremen for binding to Dkk-1.

Alternatively, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of the amino acid residues R197 or K232 of Dkk-1) and/or (ii) it competes with Kremen for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or (ii) an amino acid sequence that can compete with LRP5/6 for binding to Dkk-1.

In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with LRP5/6 or Kremen, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

It is also possible, in step b), to screen for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of the amino acid residues R197 or K232 of Dkk-1) and/or that can compete with Kremen for binding to Dkk-1; and that also (ii) encode an amino acid sequence that can bind to

and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or that can compete with LRP5/6 for binding to Dkk-1. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of LRP5/6 and/or Kremen, as applicable.

It will also be clear to the skilled person that the above methods may be performed by screening a set, collection or library of amino acid sequences that correspond to (e.g. are encoded by) the nucleic acid sequences used in the above method; and such methods form further aspects of the invention.

The invention in a further aspect provides a method for preparing and generating biparatopic amino acids of the invention which comprises at least the steps of:

a) providing a set, collection or library of nucleic acid sequences, in which each nucleic acid sequence in said set, collection or library encodes a fusion protein that comprises a first amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1 that is fused via a linker sequence to a second amino acid sequence that can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on Dkk-1 (which may be the same or different as the first antigenic determinant, part, domain or epitope on Dkk-1), in which essentially each nucleic acid sequence (or most of these) encodes a fusion protein with a different linker sequence so as to provide a set, collection or library of nucleic acid sequences encoding different fusion proteins;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on Dkk-1;

and

c) isolating the nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on Dkk-1, optionally followed by expressing the encoded amino acid sequence.

As will be clear to the skilled person, this method can be used to screen for suitable or even optimal linker lengths for linking the first and second amino acid sequence. For example, in this aspect, the first amino acid sequence may be an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of the amino acid residues R197 or K232 of Dkk-1) and/or that can compete with Kremen for binding to Dkk-1; and the

second amino acid sequence may be an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or that can compete with LRP5/6 for binding to Dkk-1 (or visa versa). The screening and selection step b) may be performed as further described above.

Another method for preparing and generating biparatopic amino acids of the invention may comprise at least the steps of:

a) providing a set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for a set, collection or library of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1;

c) ligating said set, collection or library of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 to another nucleic acid sequence that encodes an amino acid sequence that can bind to and/or has affinity for Dkk-1 (e.g. a nucleic acid sequence that encodes an amino acid sequence that competes with LRP5/6 for binding Dkk-1);

and

d) from the set, collection or library of nucleic acid sequences obtained in c), isolating the nucleic acid sequences encoding a biparatopic amino acid sequence that can bind to and/or has affinity for Dkk-1 (and e.g. further selecting for nucleic acid sequences that encode a biparatopic amino acid sequence that antagonizes with higher potency compared to the monovalent amino acid sequences), followed by expressing the encoded amino acid sequence.

The biparatopic amino acid sequence obtained in the method above, can subsequently be fused to one or more further sets, collections or libraries of nucleic acid sequences encoding amino acid sequences that can bind to and/or have affinity for Dkk-1 in order to obtain a triparatopic or multiparatopic amino acid sequence respectively.

In such a method, the set, collection or library of nucleic acid sequences encoding amino acid sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

The set, collection or library of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 can be obtained by any selection or screening method known in the art for the selection and/or screening of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 and as, for example, described in the Examples section.

Also, in such a method, the set, collection or library of nucleic acid sequences may encode a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of nucleic acid sequences may encode a set, collection or library of domain antibodies or single domain antibodies, or a set, collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of nucleic acid sequences may be an immune set, collection or library of nucleic acid sequences, for example derived from a mammal that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The set, collection or library of nucleic acid sequences may for example encode an immune set, collection or library of heavy chain variable domains or of light chain variable domains. In one specific aspect, the set, collection or library of nucleotide sequences may encode a set, collection or library of V_{HH} sequences.

In the above methods, the nucleic acid sequence may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) nucleotide sequences encoding amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

Another method for preparing and generating biparatopic amino acids of the invention may comprise at least the steps of:

a) providing a first set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said first set, collection or library of nucleic acid sequences for a nucleic acid sequence that encodes an amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1;

c) ligating the nucleic acid sequence encoding said amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1 obtained in b) to another set, collection or library of nucleic acid sequences encoding amino acid sequences to obtain a set, collection or library of nucleic acid sequences that encode fusion proteins;

d) screening said set, collection or library of nucleic acid sequences obtained in step c) for a nucleic acid sequence that encodes an amino acid sequence that can bind a second antigenic determinant, part, domain or epitope on Dkk-1 different from the first antigenic determinant, part, domain or epitope on Dkk-1;

and

e) isolating the nucleic acid sequence that encodes an amino acid sequence that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on Dkk-1, optionally followed by expressing the encoded amino acid sequence.

In a preferred aspect of the above method, the first amino acid sequence obtained in step b) is preferably such that (i) it can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or (ii) competes with LRP5/6 for binding to Dkk-1; and in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of the amino acid residues R197 or K232 of Dkk-1) and/or (ii) an amino acid sequence that can compete with Kremen for binding to Dkk-1.

Alternatively, the first amino acid sequence obtained in step b) is preferably such that (i) it can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of the amino acid residues R197 or K232 of Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1; and in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or (ii) an amino acid sequence that can compete with LRP5/6 for binding to Dkk-1.

In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with LRP5/6 or Kremen, respectively, may be performed using

generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

It is also possible, in step d), to screen for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of the amino acid residues R197 or K232 of Dkk-1) and/or that can compete with Kremen for binding to Dkk-1; and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or that can compete with LRP5/6 for binding to Dkk-1. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of LRP5/6 and/or Kremen, as applicable.

The biparatopic amino acid sequence obtained in the method above, can subsequently be fused to one or more further sets, collections or libraries of nucleic acid sequences encoding amino acid sequences that can bind to and/or have affinity for Dkk-1 in order to obtain a triparatopic or multiparatopic amino acid sequence respectively.

In such a method, the set, collection or library of nucleic acid sequences encoding amino acid sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

The set, collection or library of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 can be obtained by any selection or screening method known in the art for the selection and/or screening of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 and as, for example, described in the Examples section.

Also, in such a method, the set, collection or library of nucleic acid sequences may encode a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of nucleic acid sequences may encode a set, collection or library of domain antibodies or single domain antibodies, or a set,

collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of nucleic acid sequences may be an immune set, collection or library of nucleic acid sequences, for example derived from a mammal that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The set, collection or library of nucleic acid sequences may for example encode an immune set, collection or library of heavy chain variable domains or of light chain variable domains. In one specific aspect, the set, collection or library of nucleotide sequences may encode a set, collection or library of V_{HH} sequences.

In the above methods, the nucleic acid sequence may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) nucleotide sequences encoding amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

The invention also relates to amino acid sequences that are obtained by the above methods, or alternatively by a method that comprises one of the above methods and in addition at least the steps of determining the nucleotide sequence or amino acid sequence of said immunoglobulin sequence; and of expressing or synthesizing said amino acid sequence in a manner known per se, such as by expression in a suitable host cell or host organism or by chemical synthesis.

Another method for preparing multivalent and/or multiparatopic/biparatopic amino acids or constructs of the invention may comprise at least the steps of linking two or more monovalent amino acid sequences or monovalent construct of the invention and for example one or more linkers together in a suitable manner. The monovalent constructs (and linkers) can be coupled by any method known in the art and as further described herein. Preferred techniques include the linking of the nucleic acid sequences that encode the monovalent constructs (and linkers) to prepare a genetic construct that expresses the multivalent and/or multiparatopic/biparatopic amino acid or construct. Techniques for linking amino acid sequences or nucleic acid sequences will be clear to the skilled person, and reference is again made to the standard handbooks, such as Sambrook et al. and Ausubel et al., mentioned above, as well as the Examples below.

Accordingly, the present invention also relates to the use of a monovalent construct (which may comprise or essentially consists of an amino acid sequence of the invention such as a domain antibody, an amino acid sequence that is suitable for use as a domain antibody, a single domain antibody, an amino acid sequence that is suitable for use as a single domain antibody, a "dAb", an amino acid sequences that is suitable for use as a dAb, or a Nanobody®) in providing and/or preparing a multivalent (such as multiparatopic, and preferably biparatopic) compound or construct. The monovalent construct is then used as a binding domain or binding unit in providing and/or preparing the multivalent (such as multiparatopic, and preferably biparatopic) construct comprising two (e.g. in a biparatopic construct) or more (e.g. in a multiparatopic construct) binding units. In this respect, the monovalent construct may be used as a binding domain or binding unit in providing and/or preparing a multivalent (such as multiparatopic, and preferably biparatopic) construct of the invention comprising two or more binding units.

In a preferred aspect, the monovalent construct (which may comprise or essentially consists of an amino acid sequence of the invention such as a domain antibody, an amino acid sequence that is suitable for use as a domain antibody, a single domain antibody, an amino acid sequence that is suitable for use as a single domain antibody, a "dAb", an amino acid sequences that is suitable for use as a dAb, or a Nanobody®) is used in providing and/or preparing a multivalent (such as multiparatopic, and preferably biparatopic) construct that exhibits intramolecular binding compared to intermolecular binding. In such multivalent constructs of the invention that comprises amino acid sequences directed against two or more (different) antigenic determinants on the same antigen (for example against different epitopes of an antigen and/or against different subunits of a multimeric receptor or protein), the length and flexibility of the linker are preferably such that, when the multivalent construct binds to Dkk-1, at least two and preferably all of the amino acid sequences that are present in the multivalent construct can (simultaneously) bind to each of their intended antigenic determinants, epitopes, parts or domains, most preferably so as to allow binding with increased avidity and also intramolecular binding and/or recognition. Accordingly, the present invention also relates to the use of a monovalent construct (which may comprise or essentially consists of an amino acid sequence of the invention such as a domain antibody, an amino acid sequence that is suitable for use as a domain antibody, a single domain antibody, an amino acid sequence that is suitable for use as a single domain antibody, a "dAb", an amino acid sequences that is suitable for use as a dAb, or a Nanobody®) as a binding domain or binding unit in providing and/or preparing a multivalent (such as multiparatopic, and preferably biparatopic) construct, wherein the binding domains or binding units are linked via a linker such that the multivalent (such as

multiparatopic, and preferably biparatopic) construct preferably exhibits intramolecular binding compared to intermolecular binding.

In some of the most preferred multiparatopic (such as biparatopic) polypeptides of the invention, (i) at least one monovalent construct of the invention (and in particular at least one Nanobody®) is used that is directed against the LRP5/6 binding site on Dkk-1 (and in particular against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular against at least one of amino acid residues R242 or H210 of Dkk-1) and/or that is capable of competing with LRP5/6 for binding to Dkk-1; and at least one amino acid sequence of the invention (and in particular at least one Nanobody®) is used that is directed against another antigenic determinant, epitope, part or domain of Dkk-1. In such a preferred multiparatopic construct of the invention, the linker is most preferably such that the multiparatopic construct of the invention is capable of (simultaneously) binding to both the LRP5/6 binding site on Dkk-1 (and in particular to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular to at least one of amino acid residues R242 or H210 of Dkk-1) as well as to the other antigenic determinant, epitope, part or domain of Dkk-1, again most preferably so as to allow binding with increased avidity and also intramolecular binding and/or recognition. Accordingly, also encompassed in the present invention is the use of a monovalent construct comprising an amino acid of the invention (and in particular a Nanobody®) that is directed against the LRP5/6 binding site on Dkk-1 (and in particular against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular against at least one of amino acid residues R242 or H210 of Dkk-1) and/or that is capable of competing with LRP5/6 for binding to Dkk-1, as a binding domain or binding unit in providing and/or preparing a multiparatopic (such as biparatopic) construct, wherein the binding domains or binding units are linked via a linker such that the multiparatopic (such as biparatopic) construct preferably exhibits intramolecular binding compared to intermolecular binding.

In some of the most preferred multiparatopic polypeptides of the invention, (i) at least one monovalent construct of the invention (and in particular at least one Nanobody®) is used that is directed against the Kremen binding site on Dkk-1 (and in particular against at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular against at least one of the amino acid residues R197 or K232 of Dkk-1) and/or that is capable of competing with Kremen for binding to Dkk-1; and at least one amino acid of the invention (and in particular at least one Nanobody®) is used that is directed against another antigenic determinant, epitope, part or domain of Dkk-1. In such a preferred multiparatopic construct of the invention, the linker is most preferably such that the multiparatopic construct of the invention is capable of (simultaneously) binding to

both the Kremen binding site on Dkk-1 (and in particular to at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular to at least one of the amino acid residues R197 or K232 of Dkk-1), as well as to the other antigenic determinant, epitope, part or domain of Dkk-1, again most preferably so as to allow binding with increased avidity and also intramolecular binding and/or recognition. Accordingly, also encompassed in the present invention is the use of a monovalent construct comprising an amino acid sequence of the invention (and in particular at least one Nanobody®) that is directed against the Kremen binding site on Dkk-1 (and in particular against at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular against at least one of the amino acid residues R197 or K232 of Dkk-1) and/or that is capable of competing with Kremen for binding to Dkk-1, as a binding domain or binding unit in providing and/or preparing a multiparatopic (such as a biparatopic) construct, wherein the binding domains or binding units are linked via a linker such that the multiparatopic (such as biparatopic) construct preferably exhibits intramolecular binding compared to intermolecular binding.

In some of the most preferred multiparatopic polypeptides of the invention, (i) at least one monovalent construct of the invention (and in particular at least one Nanobody®) is used that is directed against the LRP5/6 binding site on Dkk-1 (and in particular against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular against at least one of amino acid residues R242 or H210 of Dkk-1) and/or that is capable of competing with LRP5/6 for binding to Dkk-1; and at least one monovalent construct of the invention (and in particular at least one Nanobody®) is used that is directed against the Kremen binding site on Dkk-1 (and in particular against at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular against at least one of the amino acid residues R197 or K232 of Dkk-1) and/or that is capable of competing with Kremen for binding to Dkk-1. In such a preferred multiparatopic construct of the invention, the linker is most preferably such that the multiparatopic construct of the invention is capable of (simultaneously) binding to both the LRP5/6 binding site on Dkk-1 (and in particular to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular to at least one of amino acid residues R242 or H210 of Dkk-1) as well as the Kremen binding site on Dkk-1 (and in particular to at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular to at least one of the amino acid residues R197 or K232 of Dkk-1), again most preferably so as to allow binding with increased avidity and also intramolecular binding and/or recognition. Accordingly, also encompassed in the present invention is the use of a monovalent construct comprising an amino acid sequence of the invention (and in particular at least one Nanobody®) that is directed against the LRP5/6 binding site on Dkk-1 (and in particular against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in

particular against at least one of amino acid residues R242 or H210 of Dkk-1) and/or that is capable of competing with LRP5/6 for binding to Dkk-1, and a monovalent construct comprising an amino acid of the invention (and in particular a Nanobody®) that is directed against the Kremen binding site on Dkk-1 (and in particular against at least one of the amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular against at least one of the amino acid residues R197 or K232 of Dkk-1) and/or that is capable of competing with Kremen for binding to Dkk-1, as binding domains or binding units in providing and/or preparing a multiparatopic (such as a biparatopic) construct, wherein the binding domains or binding units are linked via a linker such that the multiparatopic (such as biparatopic) construct preferably exhibits intramolecular binding compared to intermolecular binding.

In this respect, the present invention also relates to the use of a nucleic acid or nucleotide sequence that encodes a monovalent construct of the invention for the preparation of a genetic construct (as further defined herein) that encodes a multivalent (such as multiparatopic, and preferably biparatopic) construct. Also, as will be clear to the skilled person, to prepare such a genetic construct, encoding a multivalent (such as multiparatopic, and preferably biparatopic) construct of the invention, several nucleotide sequences, such as at least two nucleotide sequences encoding a monovalent construct of the invention and for example nucleic acids encoding one or more linkers can be linked together in a suitable manner. Such genetic constructs generally also comprises one or more elements of genetic constructs known per se, such as for example one or more suitable regulatory elements (such as a suitable promoter(s), enhancer(s), terminator(s), etc.) and the further elements of genetic constructs referred to herein.

Techniques for generating the nucleic acids of the invention will be clear to the skilled person and may for instance include, but are not limited to, automated DNA synthesis; site-directed mutagenesis; combining two or more naturally occurring and/or synthetic sequences (or two or more parts thereof), introduction of mutations that lead to the expression of a truncated expression product; introduction of one or more restriction sites (e.g. to create cassettes and/or regions that may easily be digested and/or ligated using suitable restriction enzymes), and/or the introduction of mutations by means of a PCR reaction using one or more "mismatched" primers. These and other techniques will be clear to the skilled person, and reference is again made to the standard handbooks, such as Sambrook et al. and Ausubel et al., mentioned above, as well as the Examples below.

The invention also relates to amino acid sequences that are obtained by the above methods, or alternatively by a method that comprises the one of the above methods and in addition at least the steps of determining the nucleotide sequence or amino acid sequence of said immunoglobulin

sequence; and of expressing or synthesizing said amino acid sequence in a manner known per se, such as by expression in a suitable host cell or host organism or by chemical synthesis.

Also, following the steps above, one or more amino acid sequences of the invention may be suitably humanized (or alternatively camelized); and/or the amino acid sequence(s) thus obtained may be linked to each other or to one or more other suitable amino acid sequences (optionally via one or more suitable linkers) so as to provide a polypeptide of the invention. Also, a nucleic acid sequence encoding an amino acid sequence of the invention may be suitably humanized (or alternatively camelized) and suitably expressed; and/or one or more nucleic acid sequences encoding an amino acid sequence of the invention may be linked to each other or to one or more nucleic acid sequences that encode other suitable amino acid sequences (optionally via nucleotide sequences that encode one or more suitable linkers), after which the nucleotide sequence thus obtained may be suitably expressed so as to provide a polypeptide of the invention.

The invention further relates to applications and uses of the amino acid sequences, compounds, constructs, polypeptides, nucleic acids, host cells, products and compositions described herein, as well as to methods for the prevention and/or treatment for diseases and disorders associated with Dkk-1. Some preferred but non-limiting applications and uses will become clear from the further description herein.

The invention also relates to the amino acid sequences, compounds, constructs, polypeptides, nucleic acids, host cells, products and compositions described herein for use in therapy.

In particular, the invention also relates to the amino acid sequences, compounds, constructs, polypeptides, nucleic acids, host cells, products and compositions described herein for use in therapy of a disease or disorder that can be prevented or treated by administering, to a subject in need thereof, of (a pharmaceutically effective amount of) an amino acid sequence, compound, construct or polypeptide as described herein.

More in particular, the invention relates to the amino acid sequences, compounds, constructs, polypeptides, nucleic acids, host cells, products and compositions described herein for use in therapy of Dkk-1-associated diseases or disorders.

Other aspects, embodiments, advantages and applications of the invention will also become clear from the further description herein, in which the invention will be described and discussed in more detail with reference to the Nanobodies® of the invention and polypeptides of the invention comprising the same, which form some of the preferred aspects of the invention.

As will become clear from the further description herein, Nanobodies® generally offer certain advantages (outlined herein) compared to "dAb's" or similar (single) domain antibodies or

immunoglobulin sequences, which advantages are also provided by the Nanobodies® of the invention. However, it will be clear to the skilled person that the more general aspects of the teaching below can also be applied (either directly or analogously) to other amino acid sequences of the invention.

Detailed description of the invention

In the present description, examples and claims:

a) Unless indicated or defined otherwise, all terms used have their usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the standard handbooks mentioned in paragraph a) on page 46 of WO 08/020079.

b) Unless indicated otherwise, the terms "immunoglobulin sequence", "sequence", "nucleotide sequence" and "nucleic acid" are as described in paragraph b) on page 46 of WO 08/020079.

c) Unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known per se, as will be clear to the skilled person. Reference is for example again made to the standard handbooks and the general background art mentioned herein and to the further references cited therein; as well as to for example the following reviews Presta, Adv. Drug Deliv. Rev. 2006, 58 (5-6): 640-56; Levin and Weiss, Mol. Biosyst. 2006, 2(1): 49-57; Irving et al., J. Immunol. Methods, 2001, 248(1-2), 31-45; Schmitz et al., Placenta, 2000, 21 Suppl. A, S106-12, Gonzales et al., Tumour Biol., 2005, 26(1), 31-43, which describe techniques for protein engineering, such as affinity maturation and other techniques for improving the specificity and other desired properties of proteins such as immunoglobulins.

d) Amino acid residues will be indicated according to the standard three-letter or one-letter amino acid code. Reference is made to Table A-2 on page 48 of the International application WO 08/020079 of Ablynx N.V. entitled *"Amino acid sequences directed against IL-6R and polypeptides comprising the same for the treatment of diseases and disorders associated with Il-6 mediated signaling"*.

e) For the purposes of comparing two or more nucleotide sequences, the percentage of *"sequence identity"* between a first nucleotide sequence and a second nucleotide sequence may be calculated or determined as described in paragraph e) on page 49 of WO 08/020079 (incorporated herein by reference), such as by dividing [*the number of nucleotides in the first nucleotide sequence that are identical to the nucleotides at the corresponding positions in the second nucleotide sequence*] by [*the total number of nucleotides in the first nucleotide sequence*] and multiplying by [*100%*], in which each deletion, insertion, substitution or

addition of a nucleotide in the second nucleotide sequence - compared to the first nucleotide sequence - is considered as a difference at a single nucleotide (position); or using a suitable computer algorithm or technique, again as described in paragraph e) on pages 49 of WO 08/020079 (incorporated herein by reference).

f) For the purposes of comparing two or more amino acid sequences, the percentage of "*sequence identity*" between a first amino acid sequence and a second amino acid sequence (also referred to herein as "*amino acid identity*") may be calculated or determined as described in paragraph f) on pages 49 and 50 of WO 08/020079 (incorporated herein by reference), such as by dividing [*the number of amino acid residues in the first amino acid sequence that are identical to the amino acid residues at the corresponding positions in the second amino acid sequence*] by [*the total number of amino acid residues in the first amino acid sequence*] and multiplying by [*100%*], in which each deletion, insertion, substitution or addition of an amino acid residue in the second amino acid sequence - compared to the first amino acid sequence - is considered as a difference at a single amino acid residue (position), i.e. as an "amino acid difference" as defined herein; or using a suitable computer algorithm or technique, again as described in paragraph f) on pages 49 and 50 of WO 08/020079 (incorporated herein by reference).

Also, in determining the degree of sequence identity between two amino acid sequences, the skilled person may take into account so-called "conservative" amino acid substitutions, as described on page 50 of WO 08/020079.

Any amino acid substitutions applied to the polypeptides described herein may also be based on the analysis of the frequencies of amino acid variations between homologous proteins of different species developed by Schulz et al., Principles of Protein Structure, Springer-Verlag, 1978, on the analyses of structure forming potentials developed by Chou and Fasman, Biochemistry 13: 211, 1974 and Adv. Enzymol., 47: 45-149, 1978, and on the analysis of hydrophobicity patterns in proteins developed by Eisenberg et al., Proc. Natl. Acad Sci. USA 81: 140-144, 1984; Kyte & Doolittle; J Molec. Biol. 157: 105-132, 198 1, and Goldman et al., Ann. Rev. Biophys. Chem. 15: 321-353, 1986, all incorporated herein in their entirety by reference. Information on the primary, secondary and tertiary structure of Nanobodies® is given in the description herein and in the general background art cited above. Also, for this purpose, the crystal structure of a V_{HH} domain from a llama is for example given by Desmyter et al., Nature Structural Biology, Vol. 3, 9, 803 (1996); Spinelli et al., Natural Structural Biology (1996); 3, 752-757; and Decanniere et al., Structure, Vol. 7, 4, 361 (1999). Further information about some of the amino acid residues that in conventional V_H domains form the V_H/V_L

interface and potential camelizing substitutions on these positions can be found in the prior art cited above.

g) Amino acid sequences and nucleic acid sequences are said to be "*exactly the same*" if they have 100% sequence identity (as defined herein) over their entire length.

h) When comparing two amino acid sequences, the term "*amino acid difference*" refers to an insertion, deletion or substitution of a single amino acid residue on a position of the first sequence, compared to the second sequence; it being understood that two amino acid sequences can contain one, two or more such amino acid differences.

i) When a nucleotide sequence or amino acid sequence is said to "comprise" another nucleotide sequence or amino acid sequence, respectively, or to "essentially consist of" another nucleotide sequence or amino acid sequence, this has the meaning given in paragraph i) on pages 51-52 of WO 08/020079.

j) The term "in essentially isolated form" has the meaning given to it in paragraph j) on pages 52 and 53 of WO 08/020079.

k) The terms "domain" and "binding domain" have the meanings given to it in paragraph k) on page 53 of WO 08/020079.

l) The terms "antigenic determinant" and "epitope", which may also be used interchangeably herein, have the meanings given to it in paragraph l) on page 53 of WO 08/020079.

m) As further described in paragraph m) on page 53 of WO 08/020079, an amino acid sequence (such as a Nanobody®, an antibody, a polypeptide of the invention, or generally an antigen binding protein or polypeptide or a fragment thereof) that can (specifically) bind to, that has affinity for and/or that has specificity for a specific antigenic determinant, epitope, antigen or protein (or for at least one part, fragment or epitope thereof) is said to be "*against*" or "*directed against*" said antigenic determinant, epitope, antigen or protein.

n) The term "*specificity*" has the meaning given to it in paragraph n) on pages 53-56 of WO 08/020079; and as mentioned therein refers to the number of different types of antigens or antigenic determinants to which a particular antigen-binding molecule or antigen-binding protein (such as a Nanobody® or a polypeptide of the invention) molecule can bind. The specificity of an antigen-binding protein can be determined based on affinity and/or avidity, as described on pages 53-56 of WO 08/020079 (incorporated herein by reference), which also describes some preferred techniques for measuring binding between an antigen-binding molecule (such as a Nanobody® or polypeptide of the invention) and the pertinent antigen. Typically, antigen-binding proteins (such as the amino acid sequences, Nanobodies® and/or polypeptides of the invention) will bind to their antigen with a dissociation constant (K_D) of 10^{-}

^{5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^{5} to 10^{12} liter/moles or more, and preferably 10^{7} to 10^{12} liter/moles or more and more preferably 10^{8} to 10^{12} liter/moles). Any K_D value greater than 10^{4} mol/liter (or any K_A value lower than 10^{4} M^{-1}) liters/mol is generally considered to indicate non-specific binding. Preferably, a monovalent immunoglobulin sequence of the invention will bind to the desired antigen with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM. Specific binding of an antigen-binding protein to an antigen or antigenic determinant can be determined in any suitable manner known per se, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, and the different variants thereof known per se in the art; as well as the other techniques mentioned herein. As will be clear to the skilled person, and as described on pages 53-56 of WO 08/020079, the dissociation constant may be the actual or apparent dissociation constant. Methods for determining the dissociation constant will be clear to the skilled person, and for example include the techniques mentioned on pages 53-56 of WO 08/020079.

o) The half-life of an amino acid sequence, compound or polypeptide of the invention can generally be defined as described in paragraph o) on page 57 of WO 08/020079 and as mentioned therein refers to the time taken for the serum concentration of the amino acid sequence, compound or polypeptide to be reduced by 50%, in vivo, for example due to degradation of the sequence or compound and/or clearance or sequestration of the sequence or compound by natural mechanisms. The in vivo half-life of an amino acid sequence, compound or polypeptide of the invention can be determined in any manner known per se, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally be as described in paragraph o) on page 57 of WO 08/020079. As also mentioned in paragraph o) on page 57 of WO 08/020079, the half-life can be expressed using parameters such as the t1/2-alpha, t1/2-beta and the area under the curve (AUC). Reference is for example made to the Experimental Part below, as well as to the standard handbooks, such as Kenneth, A et al: Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and Peters et al, Pharmacokinete analysis: A Practical Approach (1996). Reference is also made to "Pharmacokinetics", M Gibaldi & D Perron, published by Marcel Dekker, 2nd Rev. edition (1982). The terms "increase in half-life" or "increased half-life" as also as defined in paragraph o) on page 57 of WO 08/020079 and in particular refer to

an increase in the t1/2-beta, either with or without an increase in the t1/2-alpha and/or the AUC or both.

p) In the context of the present invention, "modulating" or "to modulate" generally means either reducing or inhibiting the activity of, or alternatively increasing the activity of, a target or antigen, as measured using a suitable in vitro, cellular or in vivo assay. In particular, "modulating" or "to modulate" may mean either reducing or inhibiting the activity of, or alternatively increasing a (relevant or intended) biological activity of, a target or antigen, as measured using a suitable in vitro, cellular or in vivo assay (which will usually depend on the target or antigen involved), by at least 1%, preferably at least 5%, such as at least 10% or at least 25%, for example by at least 50%, at least 60%, at least 70%, at least 80%, or 90% or more, compared to activity of the target or antigen in the same assay under the same conditions but without the presence of the construct of the invention.

As will be clear to the skilled person, "modulating" may also involve effecting a change (which may either be an increase or a decrease) in affinity, avidity, specificity and/or selectivity of a target or antigen for one or more of its ligands, binding partners, partners for association into a homomultimeric or heteromultimeric form, or substrates; and/or effecting a change (which may either be an increase or a decrease) in the sensitivity of the target or antigen for one or more conditions in the medium or surroundings in which the target or antigen is present (such as pH, ion strength, the presence of co-factors, etc.), compared to the same conditions but without the presence of the construct of the invention. As will be clear to the skilled person, this may again be determined in any suitable manner and/or using any suitable assay known per se, depending on the target or antigen involved.

"Modulating" may also mean effecting a change (i.e. an activity as an agonist, as an antagonist or as a reverse agonist, respectively, depending on the target or antigen and the desired biological or physiological effect) with respect to one or more biological or physiological mechanisms, effects, responses, functions, pathways or activities in which the target or antigen (or in which its substrate(s), ligand(s) or pathway(s) are involved, such as its signaling pathway or metabolic pathway and their associated biological or physiological effects) is involved. Again, as will be clear to the skilled person, such an action as an agonist or an antagonist may be determined in any suitable manner and/or using any suitable (in vitro and usually cellular or in assay) assay known per se, depending on the target or antigen involved. In particular, an action as an agonist or antagonist may be such that an intended biological or physiological activity is increased or decreased, respectively, by at least 1%, preferably at least 5%, such as at least 10% or at least 25%, for example by at least 50%, at least 60%, at least

70%, at least 80%, or 90% or more, compared to the biological or physiological activity in the same assay under the same conditions but without the presence of the construct of the invention.

Modulating may for example also involve allosteric modulation of the target or antigen; and/or reducing or inhibiting the binding of the target or antigen to one of its substrates or ligands and/or competing with a natural ligand, substrate for binding to the target or antigen. Modulating may also involve activating the target or antigen or the mechanism or pathway in which it is involved. Modulating may for example also involve effecting a change in respect of the folding or confirmation of the target or antigen, or in respect of the ability of the target or antigen to fold, to change its confirmation (for example, upon binding of a ligand), to associate with other (sub)units, or to disassociate. Modulating may for example also involve effecting a change in the ability of the target or antigen to transport other compounds or to serve as a channel for other compounds (such as ions).

Modulating may be reversible or irreversible, but for pharmaceutical and pharmacological purposes will usually be in a reversible manner.

q) In respect of a target or antigen, the term "interaction site" on the target or antigen means a site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen that is a site for binding to a ligand, receptor or other binding partner, a catalytic site, a cleavage site, a site for allosteric interaction, a site involved in multimerisation (such as homomerization or heterodimerization) of the target or antigen; or any other site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen that is involved in a biological action or mechanism of the target or antigen. More generally, an "interaction site" can be any site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen to which an amino acid sequence or polypeptide of the invention can bind such that the target or antigen (and/or any pathway, interaction, signaling, biological mechanism or biological effect in which the target or antigen is involved) is modulated (as defined herein).

r) An amino acid sequence or polypeptide is said to be "*specific for*" a first target or antigen compared to a second target or antigen when is binds to the first antigen with an affinity (as described above, and suitably expressed as a K_D value, K_A value, K_{off} rate and/or K_{on} rate) that is at least 10 times, such as at least 100 times, and preferably at least 1000 times, and up to 10.000 times or more better than the affinity with which said amino acid sequence or polypeptide binds to the second target or polypeptide. For example, the first antigen may bind to the target or antigen with a K_D value that is at least 10 times less, such as at least 100 times

less, and preferably at least 1000 times less, such as 10.000 times less or even less than that, than the K_D with which said amino acid sequence or polypeptide binds to the second target or polypeptide. Preferably, when an amino acid sequence or polypeptide is "specific for" a first target or antigen compared to a second target or antigen, it is directed against (as defined herein) said first target or antigen, but not directed against said second target or antigen.

s) The terms *"cross-block"*, *"cross-blocked"* and *"cross-blocking"* are used interchangeably herein to mean the ability of an amino acid sequence or other binding agents (such as a Nanobody®, polypeptide or compound or construct of the invention) to interfere with the binding of other amino acid sequences or binding agents of the invention to a given target. The extend to which an amino acid sequence or other binding agents of the invention is able to interfere with the binding of another to Dkk-1, and therefore whether it can be said to cross-block according to the invention, can be determined using competition binding assays. One particularly suitable quantitative cross-blocking assay uses a Biacore machine which can measure the extent of interactions using surface plasmon resonance technology. Another suitable quantitative cross-blocking assay uses an ELISA-based approach to measure competition between amino acid sequences or other binding agents in terms of their binding to the target.

The following generally describes a suitable Biacore assay for determining whether an amino acid sequence or other binding agent cross-blocks or is capable of cross-blocking according to the invention. It will be appreciated that the assay can be used with any of the amino acid sequences or other binding agents described herein. The Biacore machine (for example the Biacore 3000) is operated in line with the manufacturer's recommendations. Thus in one cross-blocking assay, the target protein is coupled to a CM5 Biacore chip using standard amine coupling chemistry to generate a surface that is coated with the target. Typically 200- 800 resonance units of the target would be coupled to the chip (an amount that gives easily measurable levels of binding but that is readily saturable by the concentrations of test reagent being used). Two test amino acid sequences (termed A* and B*) to be assessed for their ability to cross-block each other are mixed at a one to one molar ratio of binding sites in a suitable buffer to create the test mixture. When calculating the concentrations on a binding site basis the molecular weight of an amino acid sequence is assumed to be the total molecular weight of the amino acid sequence divided by the number of target binding sites on that amino acid sequence. The concentration of each amino acid sequence in the test mix should be high enough to readily saturate the binding sites for that amino acid sequence on the target molecules captured on the Biacore chip. The amino acid sequences in the mixture are at the

same molar concentration (on a binding basis) and that concentration would typically be between 1.00 and 1.5 micromolar (on a binding site basis). Separate solutions containing A* alone and B* alone are also prepared. A* and B* in these solutions should be in the same buffer and at the same concentration as in the test mix. The test mixture is passed over the target-coated Biacore chip and the total amount of binding recorded. The chip is then treated in such a way as to remove the bound amino acid sequences without damaging the chip-bound target. Typically this is done by treating the chip with 30 mM HCl for 60 seconds. The solution of A* alone is then passed over the target-coated surface and the amount of binding recorded. The chip is again treated to remove all of the bound amino acid sequences without damaging the chip-bound target. The solution of B* alone is then passed over the target-coated surface and the amount of binding recorded. The maximum theoretical binding of the mixture of A* and B* is next calculated, and is the sum of the binding of each amino acid sequence when passed over the target surface alone. If the actual recorded binding of the mixture is less than this theoretical maximum then the two amino acid sequences are cross-blocking each other. Thus, in general, a cross-blocking amino acid sequence or other binding agent according to the invention is one which will bind to the target in the above Biacore cross-blocking assay such that, during the assay and in the presence of a second amino acid sequence or other binding agent of the invention, the recorded binding is between 80% and 0.1% (e.g. 80% to 4%) of the maximum theoretical binding, specifically between 75% and 0.1% (e.g. 75% to 4%) of the maximum theoretical binding, and more specifically between 70% and 0.1% (e.g. 70% to 4%) of maximum theoretical binding (as just defined above) of the two amino acid sequences or binding agents in combination. The Biacore assay described above is a primary assay used to determine if amino acid sequences or other binding agents cross-block each other according to the invention. On rare occasions particular amino acid sequences or other binding agents may not bind to target coupled via amine chemistry to a CM5 Biacore chip (this usually occurs when the relevant binding site on target is masked or destroyed by the coupling to the chip). In such cases cross-blocking can be determined using a tagged version of the target, for example a N-terminal His-tagged version. In this particular format, an anti-His amino acid sequence would be coupled to the Biacore chip and then the His-tagged target would be passed over the surface of the chip and captured by the anti-His amino acid sequence. The cross blocking analysis would be carried out essentially as described above, except that after each chip regeneration cycle, new His-tagged target would be loaded back onto the anti-His amino acid sequence coated surface. In addition to the example given using N-terminal His-tagged target, C-terminal His-tagged target could alternatively be used.

Furthermore, various other tags and tag binding protein combinations that are known in the art could be used for such a cross-blocking analysis (e.g. HA tag with anti-HA antibodies; FLAG tag with anti-FLAG antibodies; biotin tag with streptavidin).

The following generally describes an ELISA assay for determining whether an amino acid sequence or other binding agent directed against a target cross-blocks or is capable of cross-blocking as defined herein. It will be appreciated that the assay can be used with any of the amino acid sequences (or other binding agents such as polypeptides of the invention) described herein. The general principal of the assay is to have an amino acid sequence or binding agent that is directed against the target coated onto the wells of an ELISA plate. An excess amount of a second, potentially cross-blocking, anti-target amino acid sequence is added in solution (i.e. not bound to the ELISA plate). A limited amount of the target is then added to the wells. The coated amino acid sequence and the amino acid sequence in solution compete for binding of the limited number of target molecules. The plate is washed to remove excess target that has not been bound by the coated amino acid sequence and to also remove the second, solution phase amino acid sequence as well as any complexes formed between the second, solution phase amino acid sequence and target. The amount of bound target is then measured using a reagent that is appropriate to detect the target. An amino acid sequence in solution that is able to cross-block the coated amino acid sequence will be able to cause a decrease in the number of target molecules that the coated amino acid sequence can bind relative to the number of target molecules that the coated amino acid sequence can bind in the absence of the second, solution phase, amino acid sequence. In the instance where the first amino acid sequence, e.g. an Ab-X, is chosen to be the immobilized amino acid sequence, it is coated onto the wells of the ELISA plate, after which the plates are blocked with a suitable blocking solution to minimize non-specific binding of reagents that are subsequently added. An excess amount of the second amino acid sequence, i.e. Ab-Y, is then added to the ELISA plate such that the moles of Ab-Y target binding sites per well are at least 10 fold higher than the moles of Ab-X target binding sites that were used, per well, during the coating of the ELISA plate. Target is then added such that the moles of target added per well are at least 25-fold lower than the moles of Ab-X target binding sites that were used for coating each well. Following a suitable incubation period the ELISA plate is washed and a reagent for detecting the target is added to measure the amount of target specifically bound by the coated anti[target amino acid sequence (in this case Ab-X). The background signal for the assay is defined as the signal obtained in wells with the coated amino acid sequence (in this case Ab-X), second solution phase amino acid sequence (in this case Ab-Y), target buffer only (i.e.

without target) and target detection reagents. The positive control signal for the assay is defined as the signal obtained in wells with the coated amino acid sequence (in this case Ab-X), second solution phase amino acid sequence buffer only (i.e. without second solution phase amino acid sequence), target and target detection reagents. The ELISA assay may be run in such a manner so as to have the positive control signal be at least 6 times the background signal. To avoid any artefacts (e.g. significantly different affinities between Ab-X and Ab-Y for the target) resulting from the choice of which amino acid sequence to use as the coating amino acid sequence and which to use as the second (competitor) amino acid sequence, the cross-blocking assay may to be run in two formats: 1) format 1 is where Ab-X is the amino acid sequence that is coated onto the ELISA plate and Ab-Y is the competitor amino acid sequence that is in solution and 2) format 2 is where Ab-Y is the amino acid sequence that is coated onto the ELISA plate and Ab-X is the competitor amino acid sequence that is in solution. Ab-X and Ab-Y are defined as cross-blocking if, either in format 1 or in format 2, the solution phase anti-target amino acid sequence is able to cause a reduction of between 60% and 100%, specifically between 70% and 100%, and more specifically between 80% and 100%, of the target detection signal (i.e. the amount of target bound by the coated amino acid sequence) as compared to the target detection signal obtained in the absence of the solution phase anti-target amino acid sequence (i.e. the positive control wells).

t) An amino acid sequence is said to be *"cross-reactive"* for two different antigens or antigenic determinants (such as serum albumin from two different species of mammal, such as human serum albumin and cyno serum albumin) if it is specific for (as defined herein) both these different antigens or antigenic determinants.

u) By binding that is *"essentially independent of the pH"* is generally meant herein that the association constant (K_A) of the amino acid sequence with respect to the serum protein (such as serum albumin) at the pH value(s) that occur in a cell of an animal or human body (as further described herein) is at least 5%, such as at least 10%, preferably at least 25%, more preferably at least 50%, even more preferably at least 60%, such as even more preferably at least 70%, such as at least 80% or 90% or more (or even more than 100%, such as more than 110%, more than 120% or even 130% or more, or even more than 150%, or even more than 200%) of the association constant (K_A) of the amino acid sequence with respect to the same serum protein at the pH value(s) that occur outside said cell. Alternatively, by binding that is *"essentially independent of the pH"* is generally meant herein that the k_{off} rate (measured by Biacore) of the amino acid sequence with respect to the serum protein (such as serum albumin) at the pH value(s) that occur in a cell of an animal or human body (as e.g. further

described herein, e.g. pH around 5.5, e.g. 5.3 to 5.7) is at least 5%, such as at least 10%, preferably at least 25%, more preferably at least 50%, even more preferably at least 60%, such as even more preferably at least 70%, such as at least 80% or 90% or more (or even more than 100%, such as more than 110%, more than 120% or even 130% or more, or even more than 150%, or even more than 200%) of the k_{off} rate of the amino acid sequence with respect to the same serum protein at the pH value(s) that occur outside said cell, e.g. pH 7.2 to 7.4. By "*the pH value(s) that occur in a cell of an animal or human body*" is meant the pH value(s) that may occur inside a cell, and in particular inside a cell that is involved in the recycling of the serum protein. In particular, by "*the pH value(s) that occur in a cell of an animal or human body*" is meant the pH value(s) that may occur inside a (sub)cellular compartment or vesicle that is involved in recycling of the serum protein (e.g. as a result of pinocytosis, endocytosis, transcytosis, exocytosis and phagocytosis or a similar mechanism of uptake or internalization into said cell), such as an endosome, lysosome or pinosome.

v) As further described herein, the total number of amino acid residues in a Nanobody® can be in the region of 110-120, is preferably 112-115, and is most preferably 113. It should however be noted that parts, fragments, analogs or derivatives (as further described herein) of a Nanobody® are not particularly limited as to their length and/or size, as long as such parts, fragments, analogs or derivatives meet the further requirements outlined herein and are also preferably suitable for the purposes described herein;

w) As further described in paragraph q) on pages 58 and 59 of WO 08/020079 (incorporated herein by reference), the amino acid residues of a Nanobody® are numbered according to the general numbering for V_H domains given by Kabat et al. ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, MD, Publication No. 91), as applied to V_{HH} domains from Camelids in the article of Riechmann and Muyldermans, J. Immunol. Methods 2000 Jun 23; 240 (1-2): 185-195 (see for example Figure 2 of this publication), and accordingly FR1 of a Nanobody® comprises the amino acid residues at positions 1-30, CDR1 of a Nanobody® comprises the amino acid residues at positions 31-35, FR2 of a Nanobody® comprises the amino acids at positions 36-49, CDR2 of a Nanobody® comprises the amino acid residues at positions 50-65, FR3 of a Nanobody® comprises the amino acid residues at positions 66-94, CDR3 of a Nanobody® comprises the amino acid residues at positions 95-102, and FR4 of a Nanobody® comprises the amino acid residues at positions 103-113.

x) The Figures, Sequence Listing and the Experimental Part/Examples are only given to further illustrate the invention and should not be interpreted or construed as limiting the scope of the

invention and/or of the appended claims in any way, unless explicitly indicated otherwise herein.

For a general description of heavy chain antibodies and the variable domains thereof, reference is inter alia made to the prior art cited herein, as well as to the prior art mentioned on page 59 of WO 08/020079 and to the list of references mentioned on pages 41-43 of the International application WO 06/040153, which prior art and references are incorporated herein by reference.

In accordance with the terminology used in the art (see the above references), the variable domains present in naturally occurring heavy chain antibodies will also be referred to as "*V_{HH} domains*", in order to distinguish them from the heavy chain variable domains that are present in conventional 4-chain antibodies (which will be referred to hereinbelow as "*V_H domains*") and from the light chain variable domains that are present in conventional 4-chain antibodies (which will be referred to hereinbelow as "*V_L domains*").

As mentioned in the prior art referred to above, V_{HH} domains have a number of unique structural characteristics and functional properties which make isolated V_{HH} domains (as well as Nanobodies® based thereon, which share these structural characteristics and functional properties with the naturally occurring V_{HH} domains) and proteins containing the same highly advantageous for use as functional antigen-binding domains or proteins. In particular, and without being limited thereto, V_{HH} domains (which have been "designed" by nature to functionally bind to an antigen without the presence of, and without any interaction with, a light chain variable domain) and Nanobodies® can function as a single, relatively small, functional antigen-binding structural unit, domain or protein. This distinguishes the V_{HH} domains from the V_H and V_L domains of conventional 4-chain antibodies, which by themselves are generally not suited for practical application as single antigen-binding proteins or domains, but need to be combined in some form or another to provide a functional antigen-binding unit (as in for example conventional antibody fragments such as Fab fragments; in ScFv's fragments, which consist of a V_H domain covalently linked to a V_L domain).

Because of these unique properties, the use of V_{HH} domains and Nanobodies® as single antigen-binding proteins or as antigen-binding domains (i.e. as part of a larger protein or polypeptide) offers a number of significant advantages over the use of conventional V_H and V_L domains, scFv's or conventional antibody fragments (such as Fab- or $F(ab')_2$-fragments), including the advantages that are listed on pages 60 and 61 of WO 08/020079.

In a specific and preferred aspect, the invention provides Nanobodies® against Dkk-1, and in particular Nanobodies® against Dkk-1 from a warm-blooded animal, and more in particular

Nanobodies® against Dkk-1 from a mammal, and especially Nanobodies® against human Dkk-1; as well as proteins and/or polypeptides comprising at least one such Nanobody®.

In particular, the invention provides Nanobodies® against Dkk-1, and proteins and/or polypeptides comprising the same, that have improved therapeutic and/or pharmacological properties and/or other advantageous properties (such as, for example, improved ease of preparation and/or reduced costs of goods), compared to conventional antibodies against Dkk-1 or fragments thereof, compared to constructs that could be based on such conventional antibodies or antibody fragments (such as Fab' fragments, $F(ab')_2$ fragments, ScFv constructs, "diabodies" and other multispecific constructs (see for example the review by Holliger and Hudson, Nat Biotechnol. 2005 Sep;23(9):1126-36)), and also compared to the so-called "dAb's" or similar (single) domain antibodies that may be derived from variable domains of conventional antibodies. These improved and advantageous properties will become clear from the further description herein, and for example include, without limitation, one or more of:

- increased affinity and/or avidity for Dkk-1, either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described hereinbelow);
- better suitability for formatting in a multivalent format (for example in a bivalent format);
- better suitability for formatting in a multispecific format (for example one of the multispecific formats described hereinbelow);
- improved suitability or susceptibility for "humanizing" substitutions (as defined herein);
- less immunogenicity, either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described hereinbelow);
- increased stability, either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described hereinbelow);
- increased specificity towards Dkk-1, either in a monovalent format, in a multivalent format (for example in a bivalent format), in a multiparatopic format (for example in a biparatopic format) and/or in a multispecific format (for example one of the multispecific formats described hereinbelow);
- decreased or where desired increased cross-reactivity with Dkk-1 from different species;

and/or

- one or more other improved properties desirable for pharmaceutical use (including prophylactic use and/or therapeutic use) and/or for diagnostic use (including but not limited

to use for imaging purposes), either in a monovalent format, in a multivalent format (for example in a bivalent format), in a multiparatopic format (for example in a biparatopic format)and/or in a multispecific format (for example one of the multispecific formats described hereinbelow).

As generally described herein for the amino acid sequences of the invention, the Nanobodies® of the invention are preferably in essentially isolated form (as defined herein), or form part of a protein or polypeptide of the invention (as defined herein), which may comprise or essentially consist of one or more Nanobodies® of the invention and which may optionally further comprise one or more further amino acid sequences (all optionally linked via one or more suitable linkers). For example, and without limitation, the one or more amino acid sequences of the invention may be used as a binding unit in such a protein or polypeptide, which may optionally contain one or more further amino acid sequences that can serve as a binding unit (i.e. against one or more other targets than Dkk-1), so as to provide a monovalent, multivalent, multiparatopic and/or multispecific polypeptide of the invention, respectively, all as described herein. In particular, such a protein or polypeptide may comprise or essentially consist of one or more Nanobodies® of the invention and optionally one or more (other) Nanobodies® (i.e. directed against other targets than Dkk-1), all optionally linked via one or more suitable linkers, so as to provide a monovalent, multivalent, multiparatopic and/or multispecific Nanobody® construct, respectively, as further described herein. Such proteins or polypeptides may also be in essentially isolated form (as defined herein).

In a Nanobody® of the invention, the binding site for binding against Dkk-1 is preferably formed by the CDR sequences. Optionally, a Nanobody® of the invention may also, and in addition to the at least one binding site for binding against Dkk-1, contain one or more further binding sites for binding against other antigens, proteins or targets. For methods and positions for introducing such second binding sites, reference is for example made to Keck and Huston, Biophysical Journal, 71, October 1996, 2002-2011; EP 0 640 130; and WO 06/07260.

As generally described herein for the amino acid sequences of the invention, when a Nanobody® of the invention (or a polypeptide of the invention comprising the same) is intended for administration to a subject (for example for therapeutic and/or diagnostic purposes as described herein), it is preferably directed against human Dkk-1; whereas for veterinary purposes, it is preferably directed against Dkk-1 from the species to be treated. Also, as with the amino acid sequences of the invention, a Nanobody® of the invention may or may not be cross-reactive (i.e. directed against Dkk-1 from two or more species of mammal, such as against human Dkk-1 and Dkk-1 from at least one of the species of mammal mentioned herein).

Also, again as generally described herein for the amino acid sequences of the invention, the Nanobodies® of the invention may generally be directed against any antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of Dkk-1. In one aspect, the Nanobodies® of the invention (and polypeptides comprising the same) are directed against the N-terminal cysteine-rich domain (amino acids 97-138). In another aspect, the Nanobodies® of the invention (and polypeptides comprising the same) are directed against the C-terminal cysteine-rich domain (amino acids 183-245), and in particular against the LRP5/6 binding site on Dkk-1 (preferably against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267, preferably against at least one of amino acid residues R242 or H210; Chen et al. 2008, J. Biol. Chem. 34: 23364) and/or capable of competing with LRP5/6 for binding to Dkk-1. Thus, in one preferred, but non-limiting aspect, the Nanobodies® of the invention (and polypeptides comprising the same) are directed against the LRP5/6 binding site onDkk-1 and/or capable of competing with LRP5/6 for binding to Dkk-1, and are as further defined herein.

In another aspect, the Nanobodies® of the invention (and polypeptides comprising the same) may also be directed against the BHQ880 binding site on Dkk-1 and/or capable of competing with BHQ880 for binding to Dkk-1. Thus, in one preferred, but non-limiting aspect, the Nanobodies® of the invention (and polypeptides comprising the same) are directed against the BHQ880 binding site onDkk-1 and/or capable of competing with BHQ880 for binding to Dkk-1, and are as further defined herein.

In yet another aspect, the Nanobodies® of the invention (and polypeptides comprising the same) may also be directed against the RH2-18 binding site on Dkk-1 and/or capable of competing with RH2-18 for binding to Dkk-1. Thus, in one preferred, but non-limiting aspect, the Nanobodies® of the invention (and polypeptides comprising the same) are directed against the RH2-18 binding site on Dkk-1 and/or capable of competing with RH2-18 for binding to Dkk-1, and are as further defined herein.

In yet another aspect, the Nanobodies® of the invention (and polypeptides comprising the same) may also be directed against the 11H10 binding site on Dkk-1 and/or capable of competing with 11H10 for binding to Dkk-1. Thus, in one preferred, but non-limiting aspect, the Nanobodies® of the invention (and polypeptides comprising the same) are directed against the 11H10 binding site onDkk-1 and/or capable of competing with 11H10 for binding to Dkk-1, and are as further defined herein.

In yet another aspect, the Nanobodies® of the invention (and polypeptides comprising the same) may also be directed against the Kremen binding site on Dkk-1 (preferably against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, preferably against at least one of amino

acid residues R197 or K232 of Dkk-1 (Wang et al. 2008, J. Biol. Chem. 283: 23371)) and/or capable of competing with Kremen for binding to Dkk-1. Thus, in one preferred, but non-limiting aspect, the Nanobodies® of the invention (and polypeptides comprising the same) are directed against the Kremen binding site onDkk-1 and/or capable of competing with Kremen for binding to Dkk-1, and are as further defined herein.

As already described herein, the amino acid sequence and structure of a Nanobody® can be considered - without however being limited thereto - to be comprised of four framework regions or "FR's" (or sometimes also referred to as "FW's"), which are referred to in the art and herein as "Framework region 1" or "FR1"; as "Framework region 2" or "FR2"; as "Framework region 3" or "FR3"; and as "Framework region 4" or "FR4", respectively; which framework regions are interrupted by three complementary determining regions or "CDR's", which are referred to in the art as "Complementarity Determining Region 1"or "CDR1"; as "Complementarity Determining Region 2" or "CDR2"; and as "Complementarity Determining Region 3" or "CDR3", respectively. Some preferred framework sequences and CDR's (and combinations thereof) that are present in the Nanobodies® of the invention are as described herein. Other suitable CDR sequences can be obtained by the methods described herein.

According to a non-limiting but preferred aspect of the invention, (the CDR sequences present in) the Nanobodies® of the invention are such that:

- the Nanobodies® can bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles);

and/or such that:

- the Nanobodies® can bind to Dkk-1 with a k_{on}-rate of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$;

and/or such that they:

- the Nanobodies® can bind to Dkk-1 with a k_{off} rate between 1 s^{-1} ($t_{1/2}$=0.69 s) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Preferably, (the CDR sequences present in) the Nanobodies® of the invention are such that: a monovalent Nanobody® of the invention (or a polypeptide that contains only one Nanobody® of the invention) is preferably such that it will bind to Dkk-1 with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

The affinity of the Nanobody® of the invention against Dkk-1 can be determined in a manner known per se, for example using the general techniques for measuring K_D, K_A, k_{off} or k_{on} mentioned herein, as well as some of the specific assays described herein.

Some preferred IC50 values for binding of the Nanobodies® of the invention (and of polypeptides comprising the same) to Dkk-1 will become clear from the further description and examples herein.

In a preferred but non-limiting aspect, the invention relates to a Nanobody® (as defined herein) against Dkk-1, which consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which:

- CDR1 is chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 335-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: [335-543;

and/or

- CDR2 is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

and/or

- CDR3 is chosen from the group consisting of:

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

or any suitable fragment of such an amino acid sequence.

In particular, according to this preferred but non-limiting aspect, the invention relates to a Nanobody® (as defined herein) against Dkk-1, which consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which:

- CDR1 is chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 335-543];

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

and

- CDR2 is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

and

- CDR3 is chosen from the group consisting of:

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

or any suitable fragment of such an amino acid sequences.

As generally mentioned herein for the amino acid sequences of the invention, when a Nanobody® of the invention contains one or more CDR1 sequences according to b) and/or c):

i) any amino acid substitution in such a CDR according to b) and/or c) is preferably, and compared to the corresponding CDR according to a), a conservative amino acid substitution (as defined herein);

and/or

ii) the CDR according to b) and/or c) preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding CDR according to a);

and/or

iii) the CDR according to b) and/or c) may be a CDR that is derived from a CDR according to a) by means of affinity maturation using one or more techniques of affinity maturation known per se.

Similarly, when a Nanobody® of the invention contains one or more CDR2 sequences according to e) and/or f):

i) any amino acid substitution in such a CDR according to e) and/or f) is preferably, and compared to the corresponding CDR according to d), a conservative amino acid substitution (as defined herein);

and/or

ii) the CDR according to e) and/or f) preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding CDR according to d);

and/or

iii) the CDR according to e) and/or f) may be a CDR that is derived from a CDR according to d) by means of affinity maturation using one or more techniques of affinity maturation known per se.

Also, similarly, when a Nanobody® of the invention contains one or more CDR3 sequences according to h) and/or i):

i) any amino acid substitution in such a CDR according to h) and/or i) is preferably, and compared to the corresponding CDR according to g), a conservative amino acid substitution (as defined herein);

and/or

ii) the CDR according to h) and/or i) preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding CDR according to g);

and/or

iii) the CDR according to h) and/or i) may be a CDR that is derived from a CDR according to g) by means of affinity maturation using one or more techniques of affinity maturation known per se.

It should be understood that the last three paragraphs generally apply to any Nanobody® of the invention that comprises one or more CDR1 sequences, CDR2 sequences and/or CDR3 sequences according to b), c), e), f), h) or i), respectively.

Of the Nanobodies® of the invention, Nanobodies® comprising one or more of the CDR's explicitly listed above are particularly preferred; Nanobodies® comprising two or more of the CDR's explicitly listed above are more particularly preferred; and Nanobodies® comprising three of the CDR's explicitly listed above are most particularly preferred.

Some particularly preferred, but non-limiting combinations of CDR sequences, as well as preferred combinations of CDR sequences and framework sequences, are mentioned in Table B-1 below, which lists the CDR sequences and framework sequences that are present in a number of preferred (but non-limiting) Nanobodies® of the invention. As will be clear to the skilled person, a combination of CDR1, CDR2 and CDR3 sequences that occur in the same clone (i.e. CDR1, CDR2 and

CDR3 sequences that are mentioned on the same line in Table B-1) will usually be preferred (although the invention in its broadest sense is not limited thereto, and also comprises other suitable combinations of the CDR sequences mentioned in Table B-1). Also, a combination of CDR sequences and framework sequences that occur in the same clone (i.e. CDR sequences and framework sequences that are mentioned on the same line in Table B-1) will usually be preferred (although the invention in its broadest sense is not limited thereto, and also comprises other suitable combinations of the CDR sequences and framework sequences mentioned in Table B-1, as well as combinations of such CDR sequences and other suitable framework sequences, e.g. as further described herein).

Also, in the Nanobodies® of the invention that comprise the combinations of CDR's mentioned in Table B-1, each CDR can be replaced by a CDR chosen from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the mentioned CDR's; in which:

i) any amino acid substitution in such a CDR is preferably, and compared to the corresponding CDR sequence mentioned in Table B-1, a conservative amino acid substitution (as defined herein);

and/or

ii) any such CDR sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding CDR sequence mentioned in Table B-1;

and/or

iii) any such CDR sequence is a CDR that is derived by means of a technique for affinity maturation known per se, and in particular starting from the corresponding CDR sequence mentioned in Table B-1.

However, as will be clear to the skilled person, the (combinations of) CDR sequences, as well as (the combinations of) CDR sequences and framework sequences mentioned in Table B-1 will generally be preferred.

Table B-1: Preferred combinations of CDR sequences, preferred combinations of framework sequences, and preferred combinations of framework and CDR sequences.

("ID" refers to the SEQ ID NO as used herein)

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
PDKKPMP4E4	125	EVQLVESGGGLVQAGG SLRLSCAASGFTFD	335	DYAIG	544	WFRQTPGK EREGVS	753	CISSSDGSTYY ADSVKG	962	RFTISSDNAKNTVYLQM NSLKPEDTAVYYCAA	1171	EVPSSCGGYSGS YYYTDDELDF	1380	WGQGTQVTVSS
PDKKPMP16A12	127	EVQLVESGGGLVQAGG SLRLSCAASGRTFN	336	NYAMG	545	WFRQTPGK EREFVA	754	AISWIIGSTYY ADSVKG	963	RFTISRDNAKNTVYLQM NGLKPEDTAVYYCAA	1172	EGGYSGTYYYTG DFDS	1381	WGQGTQVTVSS
PDKKPMP1A4	128	EVQLVESGGGSVQAGG SLRLSCAVSGRTFS	337	SYSMG	546	WFRQAPGK EREFVA	755	AISWSGGSTY YADSVKG	964	RFTISRDNAKNTVYLQM NSLKPADTAVYYCAT	1173	DESRLYGGKRPK MLYDY	1382	WGQGTQVTVSS
PDKKPMP1A8	129	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	338	SYAMG	547	WFRQAPGK EREFVA	756	AISWSGGSTV YTDSVKG	965	RFTISKDNAKNTVYLQM NSLKPEDTAVYYCAA	1174	GNGGRYYGHSR ARYDY	1383	WGQGTQVTVSS
PDKKPMP1A10	130	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	339	NYAMG	548	WFRQAPGK EREFVA	757	AISWSGGSTV YADSVKG	966	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1175	GNGGSYYGHSR ARYDY	1384	WGQGTQVTVSS
PDKKPMP1B3	131	EVQLVKSGGGLVQAGG SLRLSCAASGRTFS	340	NYAMG	549	WFRQAPGK EREFVS	758	AISWSGGSTV YADSVKG	967	RFTISRDNAENTVYLQM NSLKPEDTAVYYCAA	1176	GNGGSRYGHSR ARYDY	1385	WGQGTQVTVSS
PDKKPMP1B5	132	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	341	SYAMG	550	WFRQAPGK EREFVA	759	AISWSGGSTV YADSVKG	968	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1177	GNGGRTYGHSR ARYDY	1386	WGQGTQVTVSS
PDKKPMP1B9	133	EVQLVKSGGGLVQAGG SLRLSCAASGRTFS	342	NYAMG	551	WFRQAPGK EREFVA	760	AINWSGGSTI YADSVKG	969	RFTISRDNAKNTVYLQM NNLKPEDTAVYYCAA	1178	GNGGRSYGHSR ARYDY	1387	WGQGTQVTVSS
PDKKPMP5A1	134	EVQLVESGGGLVQPGG SLRLSCAASGRTFS	343	NNAVG	552	WFRQAPGK ERQFVA	761	AISWTAGTTY YADSVKG	970	RFTISRDNATNTVYLQL NSLKPEDTAVYFCAA	1179	GAGRDRGFSRA QYAY	1388	WGQGTQVTVSS
PDKKPMP5A2	135	EVQLVESGGGLVQAGG SLRLSCAASGRTLS	344	RSAMG	553	WFRQAPGK EREFVA	762	AINWSGGSTY YGDFVKG	971	RFTISRDSAKNTVYLQM NSLKPEDTAVYYCAA	1180	GNGGSYYGHSR ARYDY	1389	WGQGTQVTVSS
PDKKPMP5A8	136	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	345	NYAMG	554	WFRQAPGK ERDFVA	763	AISWSGGSTV YADSVKG	972	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1181	GNGGSYYGHSR ARYDY	1390	WGQGTQVTVSS
PDKKPMP5A12	137	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	346	NFAMG	555	WFRQAPGK EREFVA	764	AINWSGASTV YADSVKG	973	RFTISKDNAKNTVYLQM NSLKPEDTAVYYCAA	1182	GNGGSYYGHSR ARYDY	1391	WGQGTQVTVSS
PDKKPMP5B3	138	EVQLVESGGGLVQAGG SLRLSCEASGRTFS	347	NYATG	556	WFRQAPGK EREFVA	765	AISWSGGSTL YADSVKG	974	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1183	GNGGRNYGHSR ARYDY	1392	WGQGTQVTVSS
PDKKPMP5D11	139	KVQLVESGGGLVQAGG SLRLSCAASGRTFS	348	NYAMG	557	WFRQAPGK EREFVA	766	AISWSGASAY ADSVKG	975	RFTISRDNAKNTVYLQM NSLNAEDTAVYYCAA	1184	GNGGSYYGHSR ARYDY	1393	WGQGTQVTVSS
PDKKPMP5E9	140	EVQLVESGGGLVQAGG SLRLSCVVSGGTFN	349	VYAMG	558	WFRQAPGR LREIVA	767	AISRTGSSIYYA DSVKG	976	RFTISRDDAKKTVYLQM NSLKSEDTAVYYCAV	1185	DGRANGERPAV AGYNY	1394	WGQGTQVTVSS
PDKKPMP5G4	141	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	350	NYAMG	559	WFRQAPGK EREFVG	768	AINWSGGSTI YADSVKG	977	RFTISRDNAENTVYLQM NSLRPEDTAVYYCAA	1186	GNGGRNYGHSR ARYEY	1395	WGQGIQVTVSS

Table B-1: Continued

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
PDKKPMP10A5	142	EVQLVESGGGLVQAGG SLRLSCAASGFTFD	351	DYAIG	560	WFRQAPGK EREGVS	769	CLSSSDGSTYY ADSVKG	978	RFTISIDNAKNTVYLQM NSLKPEDTAVYYCAA	1187	VEPAHCAYQSG VTAYEIYGMDY	1396	WGKGTLVTVSS
PDKKPMP10A6	143	EVQLVESGGGLVQAGG SLRLSCVVSGGTFN	352	VYAMG	561	WFRQAPGK LREIVA	770	AISRTGSSIYYA DSVKG	979	RFTISRDDAKKTVYLQM NSLKSEDTAVYYCAV	1188	DGRANGERPAV AGYNY	1397	WGQGTQVTVSS
PDKKPMP10A1 1	144	EVQLVESGGGLVQAGG SLRLSCVVSGGTFS	353	VYAMG	562	WFRQAPGK LREIVA	771	AISRTGSSIYYT DSVKG	980	RFTISRDDAKKTVYLQM NSLKSEDTAVYYCAV	1189	DGRANGERPAV AGYNY	1398	WGQGTQVTVSS
PDKKPMP10B1	145	EVQLVKSGGGLVQAGG SLRLSCAASGRTFS	354	SYAMG	563	WFRQAPGK EREFVA	772	AINWSGSSTV YADSVKG	981	RFTISRDNAENTVYLQM NSLKPEDTAVYYCAA	1190	GNGGRNYGHSR ARYDY	1399	WGQGTQVTVSS
PDKKPMP10B4	146	EVQLVESGGGLVQAGA SLRLSCAASGGTFS	355	RYPMG	564	WFRQAPGK EREFVA	773	AISWNGGSTI YADSVKG	982	RFTISKNNAKNTVYLQM NSLRPEDTAVYYCAA	1191	GNGGSYYGHSR ARYDY	1400	WGQGTQVTVSS
PDKKPMP10C1 2	147	EVQLVESGGGLVRAGG SLRLSCVVSGGTFS	356	VYAMA	565	WFRQAPGK LREIVA	774	AISRTGSSIYYT DSVKG	983	RFTISRDDAKKTVYLQM NSLKSEDTAVYYCAI	1192	DGRANGERPAV AGYNY	1401	WGQGTQVTVSS
PDKKPMP15A9	148	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	357	SYAMG	566	WFRQAPGK EREFVA	775	AISSSASSTVY ADSVKG	984	RFAISRDNAKSTVYLQM NSLKPEDTAVYYCAA	1193	GNGGRKYGHHR ARYDY	1402	WGQGTQVTVSS
PDKKPMP15B6	149	EVQLVESRGGLVQAGG SLRLSCVASGRTFS	358	SYAMG	567	WFRQAPGK EREFVA	776	AISRSGGSTVY ADSVKG	985	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1194	GNGGRLYGHSR ARYDY	1403	WGQGTQVTVSS
PDKKPMP15F1	150	EVQLVKSGGGLVQAGG SLRLSCAASGRTFS	359	NYAMG	568	WFRQAPGK EREFVG	777	AINWSGGSTI YADSVKG	986	RFTISRDNAENTVYLQM NSLKPEDSAVYYCAA	1195	GNGGRNYGHSR ARYEY	1404	WGQGTQVTVSS
PDKKPMP16A1 1	151	EVQLVESGGGLVQPGG SLRLSCAASGRTFS	360	NYAMG	569	WFRQAPGK EREFVA	778	AISWSGGSTV YADSVKG	987	RFTISRDNAKNMVYLQ MNSLKPEDTAVYYCAA	1196	GNGGRYYNHSR TRYEY	1405	WGQGTQVTVSS
PDKKPMP1A3	152	EVQLVESGGGLVQAGG SLRLSCVVSGGTFS	361	VYAMG	570	WFRQAPGK LREIVA	779	AISRTGSSIYYA DSVKG	988	RFTISRDDAKKTVYLQM NSLKSEDTAVYYCAV	1197	DGRANGERPAV AGYNY	1406	WGQGTQVTVSS
PDKKPMP16B1	153	EVQLVKSGGGLVQAGG SLRLSCAASGRTFS	362	SYAMG	571	WFRQAPGK EREFVA	780	AISSSGSSTVY ADSVKG	989	RFAISRDNAKNTVYLQ MNNLKPEDTAVYYCAA	1198	GNGGRNYGHSR ARYDY	1407	WGQGTQVTVSS
PDKKPMP16B3	154	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	363	NYAMG	572	WFRQASGK EREFVA	781	AISWSGGSTV YADSVKG	990	RFTISRDNAKNMVYLQ MNSLRPEDTAVYYCAA	1199	GNGGRTYGHSR ARYEY	1408	WGQGTQVTVSS
PDKKPMP16B1 1	155	EVQLVESGGGLVQSGG SLRLSCVASGSTLD	364	SYDIG	573	WFRQVPGK EREGIS	782	CIRTSEDRTYY SDSVKD	991	RFTISRDNTKNTVYLQM NSLKPEDTAIYYCAT	1200	HLPLIASRLIWDS RCTLVEHEYDS	1409	WGQGTQVTVSS
PDKKPMP16B1 2	156	EVQLVESGGGLVQAGG SLRVSCAASGRTFS	365	SYAMG	574	WFRQAPGK EREFVA	783	AISWSGGSTV YADSVKG	992	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAT	1201	DESRLYGGKRPK MLYDY	1410	WGQGTQVTVSS
PDKKPMP16A3	157	EVQLVESGGGLVQAGG SLGLSCAASGRTFS	366	RYAMG	575	WFRQAPGK EREFVA	784	AISWSGGSAV YADSVKG	993	RFTISRDNAENTVYLQM NSLKSEDTALYYCAA	1202	GNGGSYYGHSR ARYDY	1411	WGQGTQVTVSS
PDKKPMP18A4	158	EVQLVESGGGLVQAGG SLRLSCAASGRAFS	367	NYAMG	576	WFRQAPGK EREFVA	785	AISWSGGSTV YADSVKG	994	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1203	GNGGRQYGHSR ARYDY	1412	WGQGTQVTVSS
PDKKPMP18A1 0	159	EVQLVKSGGGLVQAGG SLRLSCAASGRTFS	368	NYAMG	577	WFRQAPGK EREFVA	786	AISWSGGSTL YADSVKG	995	RFTISRDNAKNTVYLQM NSLKPEDTAIYYCAA	1204	GNGGRTYGHSR ARYDY	1413	WGQGTQVTVSS
PDKKPMP18A1 2	160	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	369	SYAMG	578	WFRQAPGK EREFVA	787	AISSSGSSTVY ADSVKG	996	RFAISRDNAKNTVYLQ MNNLKPEDTAVYYCAA	1205	GNGGRNYGHSR ARYDY	1414	WGQGTQVTVSS

Table B-1: Continued

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
PDKKPMP18B3	161	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	370	NYAMG	579	WFRQASGK EREFVA	788	AISWTGGSTV YADSVKG	997	RFTISRDNAKNLLYLQM NSLKPEDTAVYYCAA	1206	GNGGRTYGHSR ARYEY	1415	WGQGTQVTVSS
PDKKPMP18B4	162	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	371	NYPMG	580	WFRQAPEK EREFVA	789	AISWSGGSTA YADSVKG	998	RFTISRDNAENTVYLQM NSLKPEDTAVYYCAA	1207	GNGGRNYGHSR ARYDY	1416	WGQGTQVTVSS
PDKKPMP18B5	163	EVQLVESGGGLVQAGG SLRLSCAASGFPLD	372	DYAIG	581	WFRQAPGK EREGVS	790	CISSSDGRAVY ADSVKG	999	RFTISIDNAKNTVYLQM NSLKPEDTAVYYCAA	1208	DPYYSGSYYYTD PDRYDY	1417	WGQGTQVTVSS
PDKKPMP18E4	164	EVQLVESGGGLVQAGG SLRLSCAASGRTIS	373	NYAMG	582	WFRQAPGK EREFVA	791	AISWSGGSTIY ADSVKG	1000	RFTISRDNAKNTVSLQM NSLKPEDTAVYYCAA	1209	GNGGSYYGHSR ARYDY	1418	WGQGTQVTVSS
PDKKPMP18E6	165	EVQLVESGGGLVQAGG SLRLSCAASGFTFD	374	DYAIG	583	WFRQAPGK EGEGVS	792	CISSSDGSTYY ADSVKG	1001	RFTISSDNAKNTVYLQM NSLKPEDTAVYYCAA	1210	EYPSSCASGSYYY RDSENYDY	1419	WGQGTQVTVSS
PDKKPMP1A1	166	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	375	SYAMG	584	WFRQAPEK EREFVA	793	AISWSGGSTV YADSVKG	1002	RFTISRDNAENTVYLQM NSLKPEDTAVYYCAA	1211	GNGGRNYGHSR ARYDY	1420	WGQGTQVTVSS
PDKKPMP1B11	167	EVQLVESGGGLVRPGG SLRLSCASSASISV	376	FDAMG	585	WYRQAPGK QRERVA	794	EIINDGTTNYA GDVKG	1003	RFTISRDNTKNTMYLQ MNNLKPEDTAVYYCSA	1212	YYTDYDEALEET RGSYDY	1421	WGQGTQVTVSS
PDKKPMP5B11	168	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	377	NAAMG	586	WFRQAPGK EREFVA	795	AISWSGGSTV YADSVKG	1004	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1213	GNGGSYSGHSR ARYDY	1422	WGQGTQVTVSS
PDKKPMP5D3	169	QVQLVESGGGLVQAGG SLRLSCAVSGRTFS	378	SYTMG	587	WFRQAPGK EREFVA	796	AISWSGGSTV YADSVKG	1005	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAT	1214	DESRLYGGKRPK MLYDY	1423	WGQGTQVTVSS
PDKKPMP16A9	170	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	379	TYAMG	588	WFRQAPGK ERELVA	797	AISWSGGSTY YADSVKG	1006	RFTISRDNAKNTVDLQ MNSLKPEDTAVYYCAA	1215	GNGGSKYGHSR ARYDY	1424	WGQGTQVTVSS
PDKKPMP18A9	171	EVQLVESGRGLVQAGG SLRLSCAASGRTFS	380	SYAMG	589	WFRQAPGK EREFVA	798	AINWSGGSTV YADSVKG	1007	RFTISRDNANNTVHLQ MNSLKPEDTAVYYCAA	1216	GNGGRAYGYSR ARYEY	1425	WGQGTQVTVSS
PDKKPMP2A1	172	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	381	NYAMA	590	WFRQAPGK EREFVA	799	AISWALTRTH YADSVKG	1008	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1217	APRPKWISVRYF STSSNYDY	1426	WGQGTQVTVSS
PDKKPMP2A3	173	EVQLVESGGGLVQAGG SLGLSCTASGRAFS	382	GSAVG	591	WFRQGPG KEREFVT	800	AINWSGARTY YVDSVKG	1009	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1218	ARDRYARVLTDY EYVW	1427	WGQGTQVTVSS
PDKKPMP2A7	174	EVQLVESGGGLVQAGG SLNLSCVLSGRTLW	383	RSNMG	592	WFRQAPGK EREFVG	801	SIDWITTNIYY ADSVKG	1010	RFTISRDNPKNTFYLQM NSLKPEDTAVYRCAS	1219	SGPADARNGER WAL	1428	WGQGTQVTVSS
PDKKPMP2A10	175	EVQLVESGGGLVQAGG SLRLSCTASGSAFS	384	GTVVG	593	WFRQAPGK EREFVT	802	AINWSGARTY YADSVKG	1011	RFTISRDNAKNTAYLQM NSLRPEDTAVYYCAA	1220	GSDRYARVLTEY EYVW	1429	WGQGTQVTVSS
PDKKPMP2A11	176	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	385	NYAMA	594	WFRQAPGK EREFVA	803	AISWYLSTTYY ADSVKG	1012	RFTISRDNAKNTAFMQ MSSLKAEDTAVYYCAA	1221	APRPKRVSVRYF STSSNYDY	1430	WGQGTQVTVSS
PDKKPMP2B9	177	EVQLVESGGGLVQAGG SLRLSCVLSGRTLW	386	RSNMG	595	WFRQAPGK EREFVG	804	SIDWITTNTYY ADSVKG	1013	RFTISRDNPKNTFYLQM NSLKPEDTAVYRCAS	1222	SGPADARNGER WAL	1431	WGQGTQVTVSS
PDKKPMP3A5	178	EVQLVESGGGLVQPGG SLRLSCAASGFPFS	387	IYPMK	596	WVRQAPG KGLEWVS	805	HITRDGGGT MYADSVKG	1014	RFTISRDNAKNMLYLQ MNSLKPEDTAVYFCAR	1223	SAGKVVSRASLE NDY	1432	RGQGTQVTVSS
PDKKPMP3A9	179	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	388	NYAMA	597	WFRQAPGK EREFVA	806	AISWSLSRTHY ADSVKG	1015	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1224	APRPKWVSVRY FSTSSNYDY	1433	WGQGTQVTVSS

Table B-1: Continued

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
PDKKPMP3A11	180	EVQLVESGGGLVRAGG SLKLSCTASGRAFS	389	SSAVG	598	WFRQAPGK EREFVT	807	GINWSGARTY YADSVKG	1016	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAA	1225	GRDSHARHLSEY EYMW	1434	WGQGTQVTVSS
PDKKPMP3B1	181	EVQLVKSGGGSVQAGG SLRLSCTASGRPFD	390	NYAMA	599	WFRQAPGK EREFVA	808	AISWSLSRTHY ADSVKG	1017	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1226	APRPKRISVQYFT TSSNYDY	1435	WGQGTQVTVSS
PDKKPMP3B2	182	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	391	NYAMA	600	WFRQAPGK EREFVA	809	AISWSLSRTHY ADSVKG	1018	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1227	APRPKYVSVRYF TTSSNYDY	1436	WGQGTQVTVSS
PDKKPMP3B5	183	EVQLVESGGGLVQAGG SLRLSCAASGHTFS	392	SYAMG	601	WFRQALGK EREFVA	810	AINWSSRSTV YADSVKG	1019	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1228	GSAYVAGVRRR NAQYDY	1437	WGQGTQVTVSS
PDKKPMP3B7	184	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	393	NYAMA	602	WFRQAPGK EREFVA	811	AISWSLSRTHY ANSVEG	1020	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1229	APRPNRVSVRYF STSSNYDY	1438	WGQGTQVTVSS
PDKKPMP3D1	185	EMQLVESGGGLVQAG GSLRLSCAASGDTFS	394	NYAMG	603	WFRQAPGK EREFVA	812	AINWSSRSTV YADSVKG	1021	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1230	GTAYVAGVRRR KAEYDY	1439	WGQGTQVTVSS
PDKKPMP3F6	186	EVQLVESGGGLVQAGG SLRLSCTASGRAFS	395	SSVVG	604	WFRQAPGK EREIVT	813	AINWSGARTY YADSVKG	1022	RFTISRDNAKNTVYLQM NSLKPLDTAVYYCAA	1231	GSDRYARVLTEY EYVW	1440	WGQGTQVTVSS
PDKKPMP3G3	187	EVQLVESGGGLVQAGG SLRLSCVLSGRTLS	396	RSNMG	605	WFRQAPGK EREFVA	814	SIDWITINTYY ADSVKG	1023	RFTISRDNPKNTFYLQM NSLKPEDTAVYRCAS	1232	SGPADARNGER WHL	1441	WGQGAQVTVSS
PDKKPMP3A1	188	EVQLVELGGGLVQAGD SLTLSCTASGRTFD	397	NYAMG	606	WFRQAPGK EREFVA	815	AISWSLSRTHY ADYVKG	1024	RFTISRDNAKNTVLLQM SSLKPEDTAVYYCAA	1233	APRPKRISVSYFS TTSNYDY	1442	WGQGTQVTVSS
PDKKPMP6B2	189	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	398	NYAMA	607	WFRQAPGK EREFVA	816	AISWSLSRTHY ADSVTG	1025	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1234	APRPKWVSVRY FSTSSNYDY	1443	WGQGTQVTVSS
PDKKPMP6B3	190	EVQLVESGGGLVQAGD SLTLSCTASGRTFD	399	NYAMG	608	WFRQAPGK EREFVA	817	AISWSLSRTHY ADYVKG	1026	RFTISRDNAKNTVLLQM SSLKPEDTAVYYCAA	1235	APRPKRISVSYFS TTSNYDY	1444	WGQGTQVTVSS
PDKKPMP6C9	191	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	400	SYGMG	609	WFRQAPGK EREFVA	818	GISWTLGRTY CADSVKG	1027	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAS	1236	DGPDAYNS	1445	WGQGTQVTVSS
PDKKPMP6D1	192	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	401	NYAMA	610	WFRQAPGK EREFVG	819	AISWSLSRTHY ADSVEG	1028	RFIISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1237	APRPKWVSVRY FSTSSNYDY	1446	WGQGTQVTVSS
PDKKPMP6D2	193	EVQLVESGGGLVQPGG SLRLSCAASGFPFS	402	IYPMK	611	WVRQAPG KGLEWVS	820	HITRDGGRTM YADSVKG	1029	RFTISRDNAKNMLYLQ MNSLKPEDTAVYFCAR	1238	SAGKVVSRASLE NDY	1447	RGQGTQVTVSS
PDKKPMP6E1	194	EVQLVESGGGLVQAGG SLRLSCAASGDTFS	403	NYAMA	612	WFRQAPGK EREFVA	821	AINWSSRSTV YADSVKG	1030	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1239	GTSYVAGIRRRN AEYLY	1448	WGQGTQVTVSS
PDKKPMP6E2	195	EVQLVESGGGLVQPGG SLRLSCAASGFPFS	404	IWPMK	613	WVRQAPG KGLEWVS	822	HITRDGGRTM YADSVKG	1031	RFTISRDNAKNMLYLQ MNSLKPEDTAVYFCAR	1240	SAGKVVSRASLE NDY	1449	RGQGTQVTVSS
PDKKPMP6E10	196	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	405	NYAMA	614	WFRQAPGK EREFVA	823	AVSWSLSRTH YADSVEG	1032	RFIISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1241	APRPKWISVRYF TTSSNYDY	1450	WGQGTQVTVSS
PDKKPMP6G7	197	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	406	NYAMA	615	WFRQAPGK EREFVA	824	AISWSLSRTHY ADSVKG	1033	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1242	APRPKRISVQYFT TSSNYDY	1451	WGQGTQVTVSS
PDKKPMP13B2	198	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	407	SYGMG	616	WFRQAPGK EREFVA	825	GISWTLGRTY YADSVKG	1034	RFTISGDNAKNTVYLQ MNSLKPEDTAVYYCAS	1243	DESDAYNY	1452	WGQGTQVTVSS

Table B-1: Continued

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
PDKKPMP13B4	199	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	408	NYAMA	617	WFRQAPGK VREFVA	826	AISWSLSRTHY ADSVEG	1035	RFTISRDNAKNTAFMQ MTSLKPEDTAVYYCAA	1244	APRPKWVSVRY FSTSSNYDY	1453	WGQGTQVTVSS
PDKKPMP13B5	200	EVQLVESGGGLVQAGG SLRLSCTASGSAFS	409	SSVVG	618	WFRQAPGK EREFVT	827	AINWSGARTD YADSVKG	1036	RFTISRDNAKNTAYLQM SSLKPEDTAVYYCAA	1245	GSDRYARVLTEY EYVW	1454	WGQGTQVTVSS
PDKKPMP13B1 1	201	EVQLVKSGGGSVQAGG SLRLSCTASGRPFD	410	NYAMA	619	WFRQAPGK EREFVA	828	AISWSLSRTHY ADSVKG	1037	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1246	APRPKYVSVRYF STSSNYDY	1455	WGQGTQVTVSS
PDKKPMP13E4	202	EVQLVESGGGLVQPGG SLRLSCAASGFPFS	411	IYPLK	620	WVRQAPG KGLEWVS	829	HISRDGGRTM YADSVKG	1038	RFTISRDNAKNMLYLQ MNSLKPEDTAVFCAR	1247	SAGKVVSRASLE NDY	1456	RGQGTQVTVSS
PDKKPMP13H9	203	EVQLVESGGGLVQAGG SLRLSCAASGLTFS	412	TYAMG	621	WFRQAPGK EREFVG	830	AINWSSRSTV YADSVKG	1039	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1248	GTAVVGGVRRR KAEYDY	1457	WGQGTQVTVSS
PDKKPMP13A2	204	EVQLVESGGGWVQAG DSLRLSCTASGSAFS	413	SSVVG	622	WFRQAPGK EREFVT	831	AINWSGARTY YVDSVKG	1040	RFTISRDNAKNTAYLQM NSVKPEDTAVYYCAA	1249	GRDARARALTEY EYVW	1458	WGQGTQVTVSS
PDKKPMP13A3	205	EVQLVESGGGLVQAGG SLRLSCTASGSAFS	414	SHVVG	623	WFRQAPGK ERDFVT	832	AINWSGARTA YADSVKG	1041	RFTISRDNAKNTAYLQM NSLKPEDTAVFCAA	1250	GRDRYARDLTEY EYLW	1459	WGQGTQVTVSS
PDKKPMP13A6	206	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	415	SYGMG	624	WFRHAPGK EREFVA	833	GISWTLGRTY YADSVKG	1042	RFTISGDNAKNTVYLQ MNSLKPEDTAVYYCAS	1251	DESDAANY	1460	WGQGTQVTVSS
PDKKPMP13A7	207	EVQRVESGGGLVQAGG SLRLSCTASGSAFS	416	SSVVG	625	WFRQAPGK EREFVT	834	GINWSGARTY YADSVKG	1043	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAS	1252	GRDRYARELSEY EYVW	1461	WGQGTQVTVSS
PDKKPMP13A8	208	EVQLVESGGGLVQPGG SLRLSCAASGFPFS	417	IYPMK	626	WVRQAPG KGLEWVS	835	HITRDGERTM YADSVKG	1044	RFTISRDNAKNMLYLQ MNSLKPEDTAVFCAR	1253	SAGKVVSRASLE NDY	1462	RGQGTQVTVSS
PDKKPMP13A1 0	209	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	418	SYGMG	627	WFRPAPGK EREFVA	836	GISWTLGRTY YADSVKG	1045	RFTISGDNAKNTVYLQ MNSLKPEDTAVYYCAS	1254	DESDAYNY	1463	WGQGTQVTVSS
PDKKPMP17A4	210	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	419	SYGMG	628	WFRQAPGK EHEFVA	837	GISWTLGRTY YADSVKG	1046	RFTISGDNAKNTVYLQ MNSLKPEDTAVYYCAS	1255	DESDAYNY	1464	WGQGTQVTVSS
PDKKPMP17A5	211	EVQLVESGGGLVQAGG SLRLSCTASGRTFD	420	NYAMG	629	WFRQAPGK EREFVA	838	AISWSLSRTHY ADSVKG	1047	RFTISRDNAKNTVLLQM SSLKPEDTAVYYCAA	1256	APRPKWVSVSYF STTSNYDY	1465	WGQGTQVTVSS
PDKKPMP17A6	212	EVQLVESGGGLVQAGG SLRLSCTASGRTFD	421	NYAMG	630	WFRQSPGK AREFVA	839	AISWSLSRTHY ADSVEG	1048	RFAISRDNAKNTVLLQM SGLKPEDTAVYYCAA	1257	APRPKRVSVSYF YTSSNYDY	1466	WGQGTQVTVSS
PDKKPMP17A7	213	EVQLVESGRGLVQAGG SLRLSCTASGRAFS	422	STVVG	631	WFRQAPGK ERELVT	840	AINWSGARTY YADSVKG	1049	RFTISRDNAKNSVYLQM NSLKPEDTAVYSCAA	1258	GSDRYARVLTEY EYVW	1467	WGQGTQVTVSS
PDKKPMP17A9	214	EVQLVESGGGLVQAGG SLNLSCVLSGRTLW	423	RSNMG	632	WFRQAPGK EREFVG	841	SIDWITTNIYY ADSVKG	1050	RFTISRDNFKNTFYLQM NSLKPEDTAVYRCAS	1259	SGPADARNGDR WAL	1468	WGQGTQVTVSS
PDKKPMP17A1 0	215	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	424	NYAMA	633	WFRQAPGK EREFVA	842	AISWSLSRTHY ADSVKG	1051	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1260	APRPKWVSVRY FSNSSNYDY	1469	WGQGTQVTVSS
PDKKPMP17A1 2	216	EVQLVESGGGLVQAGG SLRLSCTASGSAFS	425	SSVVG	634	WFRQAPGK EREFVT	843	AINWSGARTY YADSVKG	1052	RFTISRENAKNTAYLQM DSLKPEDTAVYYCAA	1261	GSDRYARAFTDY EYVW	1470	WGQGTQVTVSS

Table B-1: Continued

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
PDKKPMP17B2	217	KVQLVESGGGLVQAGG SLRLSCVLSGRTLS	426	RSNMG	635	WFRQAPGK EREFVA	844	SIDWITINTYY ADSVKG	1053	RFTISRDNPKNTFYLQM NSLKPEDTAVYRCAS	1262	SGPADARNGER WHL	1471	WGQGTQVTVSS
PDKKPMP17B4	218	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	427	NYAMA	636	WFRQAPGK EREFVA	845	AISWSLSRTHY ADSVKG	1054	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1263	APRPKWVSVRY FSTSLNYDY	1472	WGQGTQVTVSS
PDKKPMP17B1 0	219	KVQLVESGGGLVQPGG SLRLSCAASGFPFS	428	IYPLK	637	WVRQAPG KGLEWVS	846	HISRDGGRTM YADSVKG	1055	RFTISRDNAKNMLYLQ MNSLKPEDTAVYFCAR	1264	SAGKVVSRASLE NDY	1473	RGQGTQVTVSS
PDKKPMP17G1 0	220	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	429	NYAMA	638	WFRQAPGK EREFVG	847	AISWSLSRTHY TDSVKG	1056	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1265	APRPKSISVRYFS TSSNYEY	1474	WGQGTQVTVSS
PDKKPMP17A2	221	KVQLVESGGGLVQAGG SLRLSCAASGHTFS	430	SYAMG	639	WFRQAPGK EREFVA	848	AINWSSRSTV YADSVKG	1057	RFTISRDNAKNTVWLQ MSSLKPEDTAVYYCAA	1266	GSAYVAGVRRR NAQYDY	1475	WGQGTQVTVSS
PDKKPMP19B1 0	222	EVQLVESGGGLVQAGD SLTLSCTASGRTFD	431	NYAMG	640	WFRQAPGK EREFVA	849	AISWSLSRTHY ADYAKG	1058	RFTISRDNAKNTVLLQM SSLKPEDTAVYYCAA	1267	APRPKRISVSYFS TTSNYDY	1476	WGQGTQVTVSS
PDKKPMP19C1	223	EVQLVESGGGLVQAGG SLRLSCTASGRTFD	432	NHAMG	641	WFRQAPGK EREFVA	850	AISWSLSRTHY ADSVSG	1059	RFTTSRDNAKNTVLLQ MSSLKPEDTAVYYCAA	1268	APRPKRVSVSYF STSSNYDY	1477	WGQGTQVTVSS
PDKKPMP19D2	224	EVQLMESGGGLVQAG GSLTLSCTASGRTFD	433	NYAMG	642	WFRQAPGK EREFVA	851	AISWSLSRTHY ADSVLG	1060	RFTISRDNAKNTVLLQM SSLKPEDTAVYYCAA	1269	APRPKRISVSYFS TSSNYDY	1478	WGQGTQVTVSS
PDKKPMP19E1	225	EVQLVESGGGLVQAGG SLNLSCTASGSAFS	434	SSVVG	643	WFRQAPGK EHEFVT	852	GINWSGARTY YADSVKG	1061	RFTISRDNAKNTAYLQM HSLKPEDTAVYYCAS	1270	GRDRYARDLSEY EYVW	1479	WGQGTQVTVSS
PDKKPMP19F5	226	EVQLVESGGGLVQAGG SLNLSCTASGNAFS	435	SSVVG	644	WFRQAPGK EHEFVT	853	GINWSGARTY YADSVKG	1062	RFTISRDNAKNTAYLQM HSLKPEDTAVYYCAS	1271	GRDRYARDLSEY EYVW	1480	WGQGTQVTVSS
PDKKPMP19A3	227	KVQLVESGGGPVQAGG SLRLSCTASGRPFD	436	NYAMA	645	WFRQAPGK EREFVA	854	AISWYVSTTFY ADSVKD	1063	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1272	APRPKRISVRYFS TSSNYDY	1481	WGQGTQVTVSS
PDKKPMP19A8	228	EVQLVESGGGLVQPGG SLRLSCAASGFPFS	437	IYPMK	646	WVRQAPG KGLEWVS	855	HITRDGGRTM YADFVKG	1064	RFTISRDNAKNMLYLQ MNSLKPEDTAVYFCAR	1273	SAGKVVSRASLE NDY	1482	RGQGTQVTVSS
PDKKPMP19B4	229	EVQLVESGGGLVQAGG SLRLSCTASGGAFS	438	STVVG	647	WFRQAPGK EREFVT	856	GINWSGARTY YADSVKG	1065	RFTISRDNAKDTAYLEM NSLKPEDTAVYYCAS	1274	GRDRYARALPDY EYLW	1483	WGQGTQVTVSS
PDKKPMP19B7	230	EVQLVESGGGLVQAGD SLTLSCTASGRTFD	439	NYAMG	648	WFRQAPGK EREFVA	857	AISWSLSRTHY ADSVKG	1066	RFTISRDNAKNTVLLQM SSLKPEDTAVYYCAA	1275	APRPKRVSVSYF STTSNYDY	1484	WGQGTQVTVSS
PDKKPMP20A8	231	EVQLVESGGGLVQPGG SLRPSCTASGSAFS	440	GSVVG	649	WFRQAPGK EREFVT	858	AINWSGARTY YADSVKG	1067	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAA	1276	ARDRYARALTEY EYLW	1485	WGQGTQVTVSS
PDKKPMP20A1 0	232	EVQLVESGGGLVQAGG SLRLSCTASGRAFS	441	SSVVG	650	WFRRAPGK EREFVT	859	GINWSGARAY YADSVKG	1068	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAS	1277	GSDRFARELSEY DYVW	1486	WGQGTQVTVSS
PDKKPMP20A1 2	233	EVQLVESGGGSVQAGG SLRLACTASGRPFD	442	NYAMA	651	WFRQAPGK EREFVA	860	AVSWSLSRTH YADSVKG	1069	RFIISRDNAKNTAFMQ MSSLEPEDTAVYYCAA	1278	APRPKWVSVRY FTTSSNYDY	1487	WGQGTQVTVSS
PDKKPMP20B1	234	KVQLVESGGGLVQAGG SLRLSCVLSGRTLS	443	RSNMG	652	WFRQAPGK EREFVA	861	SIDWITINTYY ADSVKG	1070	RFTISRDNPKNTLYLQM NSLKPEDTAVYRCAS	1279	SGPADARNGER WAL	1488	WGQGTQVTVSS

Table B-1: Continued

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
PDKKPMP20F10	235	EVQLVESGGGLVQAGGSLRLSCAASGRTFS	444	SYGMA	653	WFRQAPGKEREFVA	862	GISWTLGRTYYADSVKG	1071	RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAS	1280	DHYDTDTKSDVYNY	1489	WGQGTQVTVSS
PDKKPMP20H1	236	EVQLVESGGGLVQAGGSLRLSCIASGRAFS	445	SSVVG	654	WFRQAPGKEREFVT	863	GINWSGARTYYADSVKG	1072	RFTVSRDNAKLTAYLQMNSLKPEDTAVYYCAS	1281	GSDSRARELSRYEYVW	1490	WGQGTQVTVSS
PDKKPMP20A1	237	EVQLVESGGGSVQAGGSLRLSCTASGRPFD	446	NYAMA	655	WFRQAPGKEREFVA	864	AISWSLSRTHYAESVEG	1073	RFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA	1282	APRPKWVSVRYFSTSSNYDY	1491	WGQGTQVTVSS
PDKKPMP20A2	238	EVQLVESGGGSVQAGGSLRLSCTASGRPFD	447	DYAMA	656	WFRQAPGKEREFVA	865	AISWSLSRTHYADSVKG	1074	RFTISRDGAKNTAFMQMSSLKPEDTAVYYCAA	1283	APRPKWVSVQYFSTSANYEY	1492	WGQGTQVTVSS
PDKKPMP20A3	239	EVQLVESGGGLVQVGDSLRLSCAVSGRTLS	448	NAIMG	657	WFRQAPGKERDFVA	866	AISRSAGSLTWYPTSVKG	1075	RFTISRDNDKNTAYLQMNSLKPEDTAVYYCAA	1284	AYRFSDYSRPAGYDY	1493	WGQGTQVTVSS
PDKKPMP3A3	240	EVQLVESGGGLVQAGGSLRLSCVLSGRTFS	449	RSDMG	658	WFRQAPGKEREFVA	867	SIDWITINTYYADSVKG	1076	RFTISRDNPKNTFYLQMNSLKPEDTAVYHCAS	1285	SGPADARNVQRWAL	1494	WCQGTQVTVSS
PDKKPMP3A6	241	EVQLVESGGGLVQAGGSLRLSCTASGRPFD	450	NYAMA	659	WFRQAPGKEREFVA	868	AISWSLTRTHYADSVKG	1077	RFTISRDNAKNTALMQMSSLKPEDTAVYYCAA	1286	APRPKWISVSYFSTSSNYDY	1495	WGQGTQVTVSS
PDKKPMP3A8	242	EVQLVESGGGSVQAGGSLRLSCTASGRPFN	451	NYAMA	660	WFRQAPGKEREFVA	869	AINWSLSRTHYADSVKG	1078	RFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA	1287	APRPKCVSVRYFSTSSNYDY	1496	WGQGTQVTVSS
PDKKPMP3A12	243	EVQLVESGGGLVQAGGSLRLSCTASGRTFS	452	TSAMA	661	WFRQAPVKEREFVA	870	AVIGSSGITDYADFVKG	1079	RFTIFIDNAKNTAYLQMNSLKPEDTAVYYCAA	1288	GFGSTSILRTQDDYDY	1497	WGQGTQVTVSS
PDKKPMP3B4	244	EVQLVESGGGLVQPGGSLRLSCAASGFPFS	453	IYPMK	662	WVRQAPGKGLEWVS	871	HITRDGGRTMYADSVKG	1080	RFTISRDNAMNMLYLQMNSLKPEDTAVYFCAR	1289	SAGKVVSRASLENDY	1498	RGQGTQVTVSS
PDKKPMP6A7	245	EVQLMESGGGLVQAGGSLRLSCAASGRTFS	454	RYAMG	663	WFRQAPGKEREFVA	872	AISWSGGSIYYADSVKG	1081	RFTVSRDNANNTVYLQMNSLKPEDTAVYYCAT	1290	DSARFSSGERRSRMQYDF	1499	WGQGTQVTVSS
PDKKPMP1A2	246	EVQLVESGGGLVQAGGSLRLSCAASERTFT	455	NYAMG	664	WFRQAPGKEREFVA	873	AISWSGESTLYADSVKG	1082	RFTISRDNAKNTVYLQMNSLKPEDTAVYFCAA	1291	GNGGRNYGHSRARYDY	1500	WGQGTQVTVSS
PDKKPMP4F8	247	EVQLVESGGGLVQAGGSLRLSCAASGFTFD	456	DYAIG	665	WFRQAPGKERDGVS	874	CISSSDGSTYYADSVKG	1083	RFTISSDNAKNTVYLQMNSLKPEDTAVYYCAV	1292	EVPSSCGYYRGSYYYTDEEFAY	1501	WGQGTQVTVSS
PDKKPMP7A4	248	EVQLVESGGGLVQAGGSLRLSCAASGFTFE	457	NYAMG	666	WFRQAPGKEREGVS	875	CISSSDGSTYYADSVKG	1084	RFTISSDNTKNTVYLQMNSLKPEDTAVYYCAA	1293	EVPSSCGYYSGNYDYTDDEYDF	1502	WGQGTQVTVSS
PDKKPMP7B12	249	EVQLVESGGGLVQAGGSLRLSCAASGFTFE	458	NYAMG	667	WFRQAPGKEREGVS	876	CISSSDGRTYYADSVKG	1085	RFTISSDNTKNTVYLQMNSLKPEDTAVYYCAA	1294	EVPSSCGYYSGNYDYTDDEYDF	1503	WGQGTQVTVSS
PDKKPMP12G3	250	EVQLVESGGGLVQAGGSLRLSCAASGFTFD	459	DYAIG	668	WFRQAPGKERDGVS	877	CISSSDGSTYYADSVKG	1086	RFTISSDNAKNTVYLQMNSLKPEDTAVYYCAV	1295	EVPSSCGYYRGSSYYTDEEFAY	1504	WGQGTQVTVSS
PDKKPMP12F12	251	EVQLVESGGGLVQAGGSLRLSCAASGFTFE	460	NYAMG	669	WFRQAPGKEPEGVS	878	CISSSDGSTYYADSVKG	1087	RFTISSDNTKNTVYLQMNSLKPEDTAVYYCAA	1296	EVPSSCGYYSGNYDYTDDEYDF	1505	WGQGTQVTVSS
PDKKPMP14H2	252	EVQLVESGGGLVQAGGSLRLSCAASGFTFD	461	DYAIG	670	WFRQAPGKERDGVS	879	CISSSDGSTYYADSVKG	1088	RFTISSDNAKNTVYLQMNSLKPEDTAVYYCAV	1297	EVPSSCGYYRGSSYYTDEELAY	1506	WGQGTQVTVSS

Table B-1: Continued

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
Ctr-1D11	253	EVQLVESGGGLVQPGG SLRLSCAASGFTFS	462	NYGMS	671	WVRQAPG KGLEWVS	880	RINSGGDIINY VGSVKG	1089	RFTISRDNYKNILYLQM NGLKPEDTAIYYCAK	1298	SRDIYGWSPKDY	1507	RGQGTQVTVSS
DKKPMP13-C11A08	254	KVQLVESGGGLVQAGG SLNLSCAAHGHTFS	463	SYAMG	672	WFRQAPGK EREFVA	881	AINWSSRSTV YADSVKG	1090	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1299	GTAYVNGVRRR KAEYDY	1508	WGQGTQVTVSS
DKKPMP13-C2A02	255	EVQLVESGGGLVEPGGS LRLSCAASGFPFS	464	IYPMK	673	WVRQAPG KGLEWVS	882	HITRDGGRTM YADSVKG	1091	RFTISRDNAKNMLYLQ MNSLKPEDTAVYFCAR	1300	SAGKVVSRASLE NDY	1509	RGQGTQVTVSS
DKKPMP13-C6A05	256	EVQLVESGGGSVQAGG SLTLSCTASGRPFD	465	NYAMA	674	WFRQAPGK EREFVA	883	AVSWSLSRTH YADSVLG	1092	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1301	APRPKWVSVRY FSTSSNYDY	1510	WGQGTQVTVSS
DKKPMP13-C8A06	257	KVQLVESGGGLVQAGG SLRLSCTASGSAFS	466	STVVG	675	WFRQAPGK ERDFVT	884	AINWSGARTA YADSVKG	1093	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAA	1302	GRDRYARDLTEY EYLW	1511	WGQGTQVTVSS
DKKPMP13-C9A07	258	EVQLVESGGGLVQAGG SLRLSCTASGSAFS	467	SSVVG	676	WFRQAPRK EREFVT	885	AINWSGARTD YADSVKG	1094	RFTISRDNAKNTAWLQ MNSLKPEDTAVYYCAA	1303	GSDRYARVLTEY EYVW	1512	WGQGTQVTVSS
DKKPMP13-D5A12	259	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	468	SYGMG	677	WFRQAPGK EHEFVA	886	GISWTLGRTY YADSVKG	1095	RFTISGDNAKNTVYLQ MNSLKPEDTAVYYCAS	1304	NESDAYNY	1513	WGQGTQVTVSS
DKKPMP13-E6B04	260	EVQLVESGGGLVQAGG SLTLSCTASGRTFD	469	NYAMG	678	WFRQAPGK EREFVA	887	AISWSLSRTHY ADSVKG	1096	RFTISRDNAKNTVLLQM SSLKPEDMAVYYCAA	1305	APRPKWISVSYF STSSNYDY	1514	WGQGTQVTVSS
DKKPMP13-E9B06	261	EVQLVESGGGLVQAGG SLRLSCTASGRAFS	470	SSVVG	679	WFRQAPGK EREFVT	888	GINWSGARTY YADSVKG	1097	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAS	1306	GRDSRARALFEY EYVW	1515	WGQGTQVTVSS
DKKPMP13-F3B11	262	EVQLVESGGGLVQAGG SLRLSCTASGSAFS	471	STVVG	680	WFRQAPGK EREFVT	889	AINWSGARTY YISSVKG	1098	RFTISRDNAKNTAYLQM DSLKPEDTAVYYCAA	1307	GSDRYARVLTEY DYVW	1516	WGQGTQVTVSS
DKKPMP13-F6B12	263	EVQLVESGGGLVQAGG SLRLSCTASGRAFS	472	SSVVG	681	WFRQAPGK EREFVT	890	GINWSGARTY YADSVKG	1099	RFTISRDNGMNTAYLQ MNRLKPEDTAAYYCAS	1308	GRDRYARALFEY DYVW	1517	WGQGTQVTVSS
DKKPMP13-F7C01	264	EVQLVESGGGLVQAGG SLNLSCALSGRTFC	473	RYSMG	682	WFRQAPGK EREFVG	891	SIDWIPANIYY ADSVKG	1100	RFTISGDNPKNTLYLQM NSLKPEDTAVYHCAS	1309	NDPADARNGER GTL	1518	WGQGTQVTVSS
DKKPMP13-F8C02	265	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	474	NYAMA	683	WFRQAPGK EREFVG	892	AISWSLSRTNY ADSVKG	1101	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1310	APRPKWVSVRY FSTSSNYDY	1519	WGQGTQVTVSS
DKKPMP13-F9C03	266	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	475	NYAMA	684	WFRQAPGK EREFVA	893	AISWSLSRTHY ADSVKG	1102	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1311	APRPKYVSVRYF STSSNYDY	1520	WGQGTQVTVSS
DKKPMP13-G11C11	267	EVQLVESGGGLVQAGG SLRLSCTASGRAFS	476	TTVVG	685	WFRQAPGK ERELVT	894	AINWSGARTY YADSVKG	1103	RFTISRDNAKNTVYTQ MNSLKPEDTGVYYCAA	1312	GRDSRARVLTEY EYVW	1521	WGQGTQVTVSS
DKKPMP13-G1C04	268	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	477	SYGMG	686	WFRQAPGK EREFVA	895	GISWTLGRTY YADSVKG	1104	RFTISGDNAKNTVYLQ MNSLKPEDTAVYFCAS	1313	DESDAYNY	1522	WGQGTQVTVSS
DKKPMP13-G7C07	269	EVQLVESGGGLVQAGG SLRLSCVASGHTFS	478	SYAMG	687	WFRQAPGK EREFVA	896	AINWSSRSLV YADSVKG	1105	RFTISRDNAKNTVYLQM NSLKLEDTAVYYCAA	1314	GSAYVAGVRRR NAEYDS	1523	WGQGTQVTVSS
DKKPMP13-G9C09	270	EVQQVESGGGLVQAG GSLRLSCTASGRAFS	479	STAVG	688	WFRQAPGK ERELVT	897	AINWSGARTY YADSVKG	1106	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1315	GSDRYARVLTDY EYVW	1524	WGQGTQVTVSS

Table B-1: Continued

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
DKKPMP13-H10D07	271	EVQLVESGGGLVRAGG SLRLSCAASGLTFS	480	SYAMG	689	WFRQAPGK EREFVA	898	AVNWSGRRE LYADSVKG	1107	RFTISRDNAKNTVYLQM DSLKPEDTAVLYCAA	1316	RENRYSFNYGDI NQYNY	1525	WGQGTQVTVSS
DKKPMP13-H4D03	272	KVQLVESGGGLVQPGG SLKLSCAASGFPFS	481	IYPMK	690	WVRQAPG KGLEWVS	899	HITRDGGRTM YADSVKG	1108	RFTISRDNAKNTLYLQM NSLKPEDTAVYFCAR	1317	SAGQVVSRASLE NDY	1526	RGQGTQVTVSS
DKKPMP17-C10E04	273	EVQLVESGGGLVQAGG SLRLSCAASGDTFS	482	NYAMG	691	WFRQAPGK EREFVA	900	AINWSSRSTV YADSVKG	1109	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1318	GASYVAGVRRR NAEYLY	1527	WGQGTQVTVSS
DKKPMP17-C11E05	274	EVQLVESEGGLVQAGG SLNLSCTASGSAFS	483	SSVVG	692	WFRQAPGK EREFVT	901	GINWSGARTY YADSVKG	1110	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAS	1319	GRDRYARELSEY EYVW	1528	WGQGTQVTVSS
DKKPMP17-C1D09	275	EVQLVESGGGLVQAGG SLRLSCTASGRAFS	484	SSAVG	693	WFRQAPGK ERELAT	902	AINWSGARTY YADSVKG	1111	RFTISRDNAKNTVYLQM NSLKLEDTAVYYCAA	1320	ASDRYARVLTEY EYVW	1529	WGQGTQVTVSS
DKKPMP17-C7E01	276	EVQLVKSGGGLVQAGG SLRLSCTASGSAFS	485	STVVG	694	WFRQAPGK ERELVT	903	AINWSGARTY YADSVKG	1112	RFTISRDNARNTAYLQM NSLKPEDTAVYYCAA	1321	GSDRYARVLTEY EYMW	1530	WGQGTQVTVSS
DKKPMP17-C8E02	277	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	486	NYAVA	695	WFRQAPGK EREFVA	904	AISWSLSRTHY ANSVEG	1113	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1322	APRPKRVSVQYF STSSNYDY	1531	WGQGTQVTVSS
DKKPMP17-D10E11	278	EVQLVKSGGGLVQAGG SLRLSCAASGRTFS	487	SYGMG	696	WFRQAPGK EREFVA	905	GISWTLGRTY YADSVKG	1114	RFTISGDNAKNTVYLQ MNSLKPEDTAVYYCAS	1323	DESDAYNY	1532	WGQGTQVTVSS
DKKPMP17-D12E12	279	EVQLVESGGGLVQAGD SLRLSCTASGSAFS	488	RSVVG	697	WFRQAPGK EREFVT	906	AINWSGARTD YADSVKG	1115	RFTISRNNAENTAYLQM NSLKFEDTAVYYCAA	1324	GWDRYARVLTE YDYVW	1533	WGQGTQVTVSS
DKKPMP17-D9E09	280	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	489	SYGMG	698	WFRQAPGK EREFVA	907	GISWTLGRTY YADSVKG	1116	RFTISGDNAKNTVYLQ MNSLKPEDTAIYYCAS	1325	DESDAYNY	1534	WGQGTQVTVSS
DKKPMP17-E1F01	281	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	490	NYAMA	699	WFRQAPGK EREFVA	908	AISWSLSRTHY ADSVKG	1117	RFTISRDNAKDTAFMQ MSSLKPEDTAVYYCAA	1326	APRPKWVSVRY FSTSLNYDY	1535	WGQGTQVTVSS
DKKPMP17-E4F03	282	EVQLVESGGGLVQAGG SLTLSCTASGRTFD	491	NYAMG	700	WFRQAPGK EREFVA	909	AINWSLSRTH YADYVKG	1118	RFTISRDNAKNTVLLQM NSLKPEDTAVYYCAS	1327	APHPKRISVSYFS TTSNYDY	1536	GGQGTQVTVSS
DKKPMP17-E5F04	283	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	492	NYAMA	701	WFRQAPGK EREFVA	910	AISWSLTRTHY AESVEG	1119	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1328	APRPKRISVRYFS TSSNYDY	1537	WGQGTQVTVSS
DKKPMP17-E6F05	284	EVQLVESGGGLVQAGG SLRLSCTASGRAFS	493	SSVVG	702	WFRQAPGK EREFVT	911	GINWSGARTY YADSVKG	1120	RFTISRDNAKNTAHLQ MNSLKREDTAVYYCAS	1329	GRDRYARVLPEY EYEW	1538	WGQGTQVTVSS
DKKPMP17-E8F07	285	EVQPVESGGGLVQAGG SLRLSCTASGSAFS	494	STVVG	703	WFRQAPGK EREIVT	912	AINWSGARTY YADSVKG	1121	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAA	1330	GSDRYARVLTEY EYVW	1539	WGQGTQVTVSS
DKKPMP17-F10G04	286	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	495	NYAMA	704	WFRQAPGE EREFVA	913	AISWALTRTH YADSVKG	1122	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1331	APRPKWISVRYF STSSNYDY	1540	WGQGTQVTVSS
DKKPMP17-F12G05	287	EVQLVESGGGSVQAGG SLRLSCTAAGRPLD	496	NYAMA	705	WFRQAPGK EREFVA	914	AISWSLSRTHY ADSVKG	1123	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1332	APRPKYVSVRYF STSSNYDY	1541	WGQGTQVTVSS
DKKPMP17-F2F12	288	EVQLVESGGGLVQAGG SLRLSCTASGSAFS	497	SHVVG	706	WFRQAPGK ERDFVT	915	AINWSGARTA YADSVKG	1124	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAA	1333	GRDRYARDLTEY EYLW	1542	WGQGTQVTVSS

Table B-1: Continued

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
DKKPMP17-F3G01	289	EVQLVQSGGGLVQAGG SLRLSCVLSGRTLW	498	RSNMG	707	WFRQAPGK EREFVG	916	SIDWITTNTYY ADSVKG	1125	RFTISRDNPKNTFFLQM NSLKPEDTAVYRCAS	1334	SGPADARNGER WAL	1543	WGQGTQVTVSS
DKKPMP17-F4G02	290	EVQLVESGGGLVQAGG SLTLPCTVSGRTFD	499	NYAMG	708	WFRQAPGK EREFVA	917	AISWSLSRTHY ADSAKG	1126	RFTISRDNAKNTVLLQM SSLKPEDTAVYYCAA	1335	APRPKRVSVSYF STASNYDY	1544	WGQGTQVTVSS
DKKPMP17-G11H01	291	EVQLVESGGGLVQAGG SLGLSCAASGHTFS	500	SYAMG	709	WFRQAPGK EREFVA	918	AINWSSRSTV YADSVKG	1127	RFTISRDNAKNTVYLQM NSLKPEDTAIYYCAA	1336	GSAYVAGVRRR NAQYDY	1545	WGQGTQVTVSS
DKKPMP17-G1G06	292	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	501	NYAMA	710	WFRQAPGK EREFVA	919	AISWSLSRTHY ADSVEG	1128	RFTISRDNAKNTAFMQ MISLKPEDTAVYYCAA	1337	APRPKRVSVRYF STSSNYDY	1546	WGQGTQVTVSS
DKKPMP17-G2G07	293	EVQLVEPGGGLVQAGG SLRLSCTASGRAFS	502	STVVG	711	WFRQAPGK EREFVT	920	GINWSGARTY YADSVKG	1129	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAS	1338	GRDQYARALPEY EYVW	1547	WGQGTQVTVSS
DKKPMP17-G3G08	294	KVQLVESGGGLVQAGD SLTLSCTASGRTF	503	DNYAMG	712	WFPQAPGK EREFVA	921	AISWSLSRTHY ADYVKG	1130	RFAISRDNAKNTVLLQM SSLKPEDTAVYYCAA	1339	APRPKRISVSYFS TTSNYDY	1548	WGQGTQVTVSS
DKKPMP17-G4G09	295	EVQLVESGGGLVQAGG SLRLSCTASGSAFS	504	SFVVG	713	WFRQAPGK ERELVT	922	AINWSGARTY YADSVKG	1131	RFTISRDNARNTAYLQM NSLKPEDTAVYYCAA	1340	GSDRYARVLTEY EYMW	1549	WGQGTQVTVSS
DKKPMP17-G5G10	296	EVQLVESGGGLVQAGG SLRLSCTASGRTFD	505	NHAMG	714	WFRQAPGK ERELVA	923	AISWSLSRTHY ADSVKG	1132	RFTISRDNAKNTALLQM SSLKPEDTAVYYCAA	1341	APRPKYVSVSYFS TSSNYDY	1550	WGQGTQVTVSS
DKKPMP17-H5H04	297	EVQLVESGGGLVRAGG SLRLSCAASGRTFS	506	SYGMG	715	WFRHAPGK EREFVA	924	GISWTLGRTY YADSVKG	1133	RFTISGDNAKNTVYLQ MNSLKPEDTAVYYCAS	1342	DESDAANY	1551	WGQGTQVTVSS
DKKPMP17-H6H05	298	EVQLVESGGGSVQAGG SLRLFCTASGRPFD	507	NYAMA	716	WFRQAPGK EREFVA	925	AISWYLTTTYY ADSVKG	1134	RFTISRDNAKNTAFMQ MSSLEPEDTAVYYCAA	1343	APRPTRVSVRYF STRSNYNY	1552	WGQGTQVTVSS
DKKPMP17-H8H06	299	EVQLVESGGGLGQPGG SLRLSCAASGRSFS	508	TYTMG	717	WFRQAPGK ERQFIA	926	AMRVGVTYY VDSVKG	1135	RFTISRDNAKNTVSLQM NSLKPEDTAVYYCAA	1344	DFVLAPGMRVS RPPNY	1553	WGQGTQVTVSS
DKKPMP19-C3H07	300	EVQLVESGGGSVQAGG SLRLSCTASGRPFD	509	NYAMA	718	WFRQAPGK EREFVA	927	AISWSLSRTHY ANSVEG	1136	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1345	APRPKRVSVQYF VTSSNYDY	1554	WGQGTQVTVSS
DKKPMP19-C6H10	301	EVQLVESGGGLVQAGG SLRLSCTASGSAFS	510	GSVVG	719	WFRQAPGK EREFVT	928	AINWSGARTY YVDSVKG	1137	RFTISRDNAKNTAHLQ MNSLKPEDTAVYYCAA	1346	ARDRYARVLTEY EYLW	1555	WGQGTQVTVSS
DKKPMP19-C8H11	302	EVQLVESGGGLVQSGG SLRLSCTASGRTFD	511	NYAMG	720	WFRQAPGK EREFVA	929	AISWALSRTH YVDSVKG	1138	RFTISRDNAKDTVQLQ MSSLKPEDTAVYYCAA	1347	APRPKRVSFSYFS TSSNYEY	1556	WGQGTQVTVSS
DKKPMP19-C9H12	303	EVQLVESGGGSVQAGG SLRLSCTASGRALS	512	SAVVG	721	WFRQAPGK EREFVT	930	AINWSGARTY YADSVKG	1139	RFTISRDNAKNTVYLQM DSLKSEDTAVYYCAS	1348	ASDRYARPLDEY EYVS	1557	WGQGTQVTVSS
DKKPMP19D12A05	304	EVQLVESGGGLVQAGG SLRLSCTASGRTFD	513	NYAMG	722	WFRQAPGK EREFVA	931	AISWSLSRTHY ADSVKG	1140	RFTISRDNAKNTVLLQM SSLKPEDTAVYYCAA	1349	APRPKWVSVSYF STTSNYEY	1558	WGQGTQVTVSS
DKKPMP19D5A03	305	EVQLVESGGGLVQAGG SLRLSCTASGRAFS	514	SSVVG	723	WFRQAPGK EREFVT	932	GINWSGARTL YADSVKG	1141	RFTISRDNAKSTAFLQM NSLKPEDTAVYYCAS	1350	GRDRYARVLSEY EYEW	1559	WGQGTQVTVSS
DKKPMP19E11A09	306	EVQLVESGGGLVQAGG SLRLSCTASGRAFS	515	STVVG	724	WFRQAPGK EREFVT	933	AINWSGARTY YAESVKG	1142	RFTISRDNAKNSAYLQM NSLKPEDTAVYYCAA	1351	GSDRYARALTEY EYVW	1560	WGQGTQVTVSS

Table B-1: Continued

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
DKKPMP19E12 A10	307	EVQLVESGGGSVQAGG SLKLSCTASGRPFN	516	NYAMA	725	WFRQAPGK EREFVA	934	AISWSLSRTHY ANSVKD	1143	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1352	APRPKRVSVHYF STSSNYDY	1561	GGQGTQVTVSS
DKKPMP19E3A 06	308	EVQLVESGGGLVQAGD SLTLSCTASGRTFD	517	NYAMG	726	WFRQAPGK EREFVA	935	AISWSLSRTHY ADYVKG	1144	RFAISRDNAKNTVLLQM SSLKPEDTAVYYCAA	1353	APRPKRISVSYFS TTSNYDY	1562	WGQGTQVTVSS
DKKPMP19E7A 07	309	EVQLVESGGGSMQAG GSLRLSCTASGRPFD	518	NYAVA	727	WFRQVPGK EREFVA	936	AISWSLSRTHY ANSVEG	1145	RFTISRDNAKNTAFMQ MSSLKPEDTAVYYCAA	1354	APRPKRASVQYF STSSNYDY	1563	WGQGTQVTVSS
DKKPMP19F12 B05	310	EVQLVESGGGLVQPGG SLRLSCVASGFTFS	519	MWPLK	728	WVRQAPG KGLEWVS	937	HITSNGDRTM YAASVKG	1146	RFTISRDNAKNTLYLQM NSLKPEDTAVYYCAR	1355	SAGQVVSRASLE NDY	1564	RGQGTQVTVSS
DKKPMP19F9B 03	311	EVQLVESGGGLVQAGG SLRLSCVLSGRTLS	520	RSNMG	729	WFRQAPGK EREFVA	938	SIDWITINTYY ADSVKG	1147	RFTISRDNPKNTFYLQM NSLKPEDTAVYRCAS	1356	AGPADARNGER WAL	1565	WGQGTQVTVSS
DKKPMP19G1B 06	312	EVQLVESGGGLVQAGG SLRLSCTASGRTFD	521	NYAMG	730	WFRQVPGK EREFVA	939	AISWSLSRTHY ADSVKG	1148	RFTISRDNAKNTVLLQM SSLKPEDTAVYYCAA	1357	APRPKRVSVSYF STSNYDY	1566	WGQGTQVTVSS
DKKPMP19G6B 07	313	EVQLVESGGGLVQAGG SLRLSCTASGRAFS	522	STVVG	731	WFRQAPGK EREFVT	940	GINWSGARTY YADSVKG	1149	RFTISRDNAKNMAYLQ MNSLKPEDTAVYYCAS	1358	GSDRYARALSEY EYVW	1567	WGQGTQVTVSS
DKKPMP19G9B 10	314	EVQLVESGGGLVQAGG SLRLSCTASGRAFS	523	SSVVG	732	WFRQAPGK EREFVT	941	GINWSGARTY YADSVKG	1150	RFTISRDNAKNTAYLQM TSLKPEDTAVYYCAS	1359	GRDAYARVLPDY EYVW	1568	WGQGTQVTVSS
DKKPMP19H7C 06	315	EVQLVESGGGLVQAGG SLRLSCTASGSAFS	524	STVVG	733	WFRQAPGK EREFVT	942	AINWSGARTY YADSVKG	1151	RFTISRDNAKNTGYLQ MNSLKPEDTAVYYCAA	1360	GSDRYARVFTEY EYVW	1569	WGQGAQVTVSS
DKKPMP19H9C 07	316	EVQLVESGGGLVQAGG SLRLSCTASGRALS	525	SSVVG	734	WFRQAPGK EREFVT	943	GINWSGARTY YADSVKG	1152	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAS	1361	GSDRYARVLSEY EYVW	1570	WGRGTQVTVSS
DKKPMP15G11	317	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	526	NYAMG	735	WFRQAPGK EREFVG	944	AINWSGGSTI YADSVKG	1153	RFTISRDNAENTVYLQM NSLKPEDTAVYYCAA	1362	GNGGRMYGHSR ARYEY	1571	WGQGTQVTVSS
DKKPMP15H4	318	QVQLVESGGGVVQAG DSLSLSCAASGRTFS	527	SYAMG	736	WFRQAPGK EREFVA	945	AISWSGGSTV YADSVKG	1154	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1363	GNGGRTYGHSR ARYDY	1572	WGQGTQVTVSS
DKKPMP16A4	319	EVQLVESGGGLVQAGG SLRLSCVVSGGTFS	528	VYAMG	737	WFRQAPGK LREIVA	946	AISRTGSSIYYA DSVKG	1155	RFTISRDDAKKTVYLQM NGLKSEDTAVYYCAV	1364	DGRANGERPAV AGYNY	1573	WGQGTQVTVSS
DKKPMP16A8	320	QVQLVESGGGLVQAGG SLRLSCAASGRTFS	529	TYAMG	738	WFRQAPGK EREIVA	947	AISGSGGSTYY ADSVKG	1156	RFTISRDNAKNTVDLQ MNSLKPEDTALYYCAA	1365	GNGGRHYGHSR ARYDY	1574	WGQGTQVTVSS
DKKPMP14B11	321	EVQLVESGGGLVQAGG SLRLSCAASGFTFE	530	NYAMG	739	WFRQAPGK EPEGVS	948	CISSSDGSTYY ADSVKG	1157	RFTISSDNTKNTVYLQM NNLKPEDTAVYYCAA	1366	EVPSSCGYYSGN YDYTDDEYDF	1575	WGQGTQVTVSS
DKKPMP13B12	322	EVQLVESGGGLVQAGG SLRLSCTASGSAFS	531	STVVG	740	WFRQAPGK EREFVT	949	AINWSGARTY YADSVKG	1158	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAA	1367	GSDRYARVLTEY EYVW	1576	WGQGTQVTVSS
DKKPMP13E3	323	EVQLVESGGGLVQAGG SLRLSCAASGGTFS	532	SYAMG	741	WFRQAPGK EREFVA	950	AINWSSRSTV YADAVKG	1159	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1368	GSAYVAGVRRR NAEYHY	1577	WGQGTQVTVSS
DKKPMP19B3	324	EVQLVESGGGLVQAGD SLRLSCTASGSAFS	533	SSVVG	742	WFRQAPGK EREFVT	951	AINWSGARTD YADSVKG	1160	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAA	1369	GSDRYARVLTEY EYVW	1578	WGQGTQVTVSS

Table B-1: Continued

Clone	ID	FR1	ID	CDR 1	ID	FR2	ID	CDR 2	ID	FR3	ID	CDR 3	ID	FR4
DKKPMP15A1	325	EVQLVESGGGLVQAGG SLRLSCAVSGRTFS	534	SYTMG	743	WFRQAPGK EREFVA	952	AISWSGGSTV YADSVKG	1161	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAT	1370	DESRLYGGKRPK MLYDY	1579	WGQGTQVTVSS
DKKPMP15A5	326	EVQLVESGGGLVQAGG SLRLSCAASGRAFS	535	NYAMG	744	WFRQAPGK EREFVA	953	AISWSGGSAV YSDSVKG	1162	RFTISRDVAKNTMYLQ MNSLKPEDTAVYYCAA	1371	GNGGRQYGHSR ARYDS	1580	WGQGTQVTVSS
DKKPMP10D7	327	EVQLVESGGGLVQAGG SLRLSCVVSGGTFS	536	VYAMA	745	WFRQAPGK LREIVA	954	AISRTGSSIYYT DSVKG	1163	RFTISRDDAKKTVYLQM NSLKSEDTAVYYCAV	1372	DGRANGERPAV AGYNY	1581	WGQGTQVTVSS
DKKPMP10F12	328	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	537	NYAMG	746	WFRQAPG NEREFVA	955	AISWSGGSTV YADSVKG	1164	RFTISRDNAENTVYLQM NSLKPEDTAVYYCAA	1373	GNGGSYYGHSR ARYDY	1582	WGQGTQVTVSS
DKKPMP10G1	329	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	538	SYAMG	747	WFRLAPGK EREFVA	956	AISWSGGSTV YADSVKG	1165	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1374	GNGGRTYGHSR ARYDY	1583	WGQGTQVTVSS
DKKPMP10G8	330	EVQLVESGGGLVQAGG SLRLSCAASGRTIS	539	NAAMG	748	WFRQAPGK EREFVA	957	AISWSGGSTV YADSVKG	1166	RFTISRDNAKNTVYLQM NSPKPEDTAVYYCAA	1375	GNGGRNYGHSR ARYDY	1584	WGQGTQVTVSS
DKKPMP15A2	331	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	540	NYAMG	749	WFRQAPGK EREFVA	958	AISWSGASTIY ADSVKG	1167	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1376	GNGGSRYGHSR ARYDY	1585	WGQGTQVTVSS
DKKPMP15B4	332	EVQLVESGGGLVQAGG SLRLSCAASGRTFS	541	SYAMG	750	WFRQAPGK EREFVA	959	AISSSGSSTVY ADSVKG	1168	RFAISRDNAKNTVYLQ MNSLKPEDTAVYYCAA	1377	GNGGRNYGHSR ARYDY	1586	WGQGTQVTVSS
DKKPMP15G1	333	KVQLVESGGGLVQAGG SLRLSCAASGRTFS	542	NYAMG	751	WFRQAPGK ERDFVA	960	AISWSGGSTV YADSVKG	1169	RFTISRDNAKNTVYLQM NSLKPEDTAVYYCAA	1378	GNGGSYYGHSR ARYDY	1587	WGQGTQVTVSS
DKKPMP6C9C11	334	EVQLVESGGGLVQAGG SLRLSCTASGSAFR	543	STVVG	752	WFRQAPGK ERDLVT	961	AINWSGARTY YADSVKG	1170	RFTISRDNAKNTAYLQM NSLKPEDTAVYYCAA	1379	GRDSYARVLTEY EYVW	1588	WGQGTQVTVSS

Thus, in the Nanobodies® of the invention, at least one of the CDR1, CDR2 and CDR3 sequences present is suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1; or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% "sequence identity" (as defined herein) with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1.

In this context, by "suitably chosen" is meant that, as applicable, a CDR1 sequence is chosen from suitable CDR1 sequences (i.e. as defined herein), a CDR2 sequence is chosen from suitable CDR2 sequences (i.e. as defined herein), and a CDR3 sequence is chosen from suitable CDR3 sequence (i.e. as defined herein), respectively. More in particular, the CDR sequences are preferably chosen such that the Nanobodies® of the invention bind to Dkk-1 with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein.

In particular, in the Nanobodies® of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 sequences listed in Table B-1 or from the group of CDR3 sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR3 sequences listed in Table B-1; and/or from the group consisting of the CDR3 sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR3 sequences listed in Table B-1.

Preferably, in the Nanobodies® of the invention, at least two of the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1 or from the group consisting of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 "amino acid difference(s)" with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1.

In particular, in the Nanobodies® of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 sequences listed in Table B-1 or from the

group of CDR3 sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR3 sequences listed in Table B-1, respectively; and at least one of the CDR1 and CDR2 sequences present is suitably chosen from the group consisting of the CDR1 and CDR2 sequences, respectively, listed in Table B-1 or from the group of CDR1 and CDR2 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table B-1; and/or from the group consisting of the CDR1 and CDR2 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table B-1.

Most preferably, in the Nanobodies® of the invention, all three CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1 or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1.

Even more preferably, in the Nanobodies® of the invention, at least one of the CDR1, CDR2 and CDR3 sequences present is suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1. Preferably, in this aspect, at least one or preferably both of the other two CDR sequences present are suitably chosen from CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences, respectively, listed in Table B-1; and/or from the group consisting of the CDR sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the corresponding sequences, respectively, listed in Table B-1.

In particular, in the Nanobodies® of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 listed in Table B-1. Preferably, in this aspect, at least one and preferably both of the CDR1 and CDR2 sequences present are suitably chosen from the groups of CDR1 and CDR2 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR1 and CDR2 sequences, respectively, listed in Table B-1; and/or from the group consisting of the CDR1 and CDR2 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table B-1.

Even more preferably, in the Nanobodies® of the invention, at least two of the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1. Preferably, in this aspect, the remaining CDR sequence present is suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences listed in Table B-1; and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the corresponding sequences listed in Table B-1.

In particular, in the Nanobodies® of the invention, at least the CDR3 sequence is suitably chosen from the group consisting of the CDR3 sequences listed in Table B-1, and either the CDR1 sequence or the CDR2 sequence is suitably chosen from the group consisting of the CDR1 and CDR2 sequences, respectively, listed in Table B-1. Preferably, in this aspect, the remaining CDR sequence present is suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences listed in Table B-1; and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with the corresponding CDR sequences listed in Table B-1.

Even more preferably, in the Nanobodies® of the invention, all three CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1.

Also, generally, the combinations of CDR's listed in Table B-1 (i.e. those mentioned on the same line in Table B-1) are preferred. Thus, it is generally preferred that, when a CDR in a Nanobody® of the invention is a CDR sequence mentioned in Table B-1 or is suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with a CDR sequence listed in Table B-1; and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with a CDR sequence listed in Table B-1, that at least one and preferably both of the other CDR's are suitably chosen from the CDR sequences that belong to the same combination in Table B-1 (i.e. mentioned on the same line in Table B-1) or are suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR sequence(s) belonging to the same combination and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with the CDR sequence(s) belonging to the same combination. The other preferences indicated in the above paragraphs also apply to the combinations of CDR's mentioned in Table B-1.

Thus, by means of non-limiting examples, a Nanobody® of the invention can for example comprise a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table B-1, a CDR2 sequence that has 3, 2 or 1 amino acid difference with one of the CDR2 sequences mentioned in Table B-1 (but belonging to a different combination), and a CDR3 sequence.

Some preferred Nanobodies® of the invention may for example comprise: (1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table B-1; a CDR2 sequence that has 3, 2 or 1 amino acid difference with one of the CDR2 sequences mentioned in Table B-1 (but belonging to a different combination); and a CDR3 sequence that has more than 80 % sequence identity with one of the CDR3 sequences mentioned in Table B-1 (but belonging to a different combination); or (2) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table B-1; a CDR2 sequence, and one of the CDR3 sequences listed in Table B-1; or (3) a CDR1 sequence; a CDR2 sequence that has more than 80% sequence identity with one of the CDR2 sequence listed in Table B-1; and a CDR3 sequence that has 3, 2 or 1 amino acid differences with the CDR3 sequence mentioned in Table B-1 that belongs to the same combination as the CDR2 sequence.

Some particularly preferred Nanobodies® of the invention may for example comprise: (1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table B-1; a CDR2 sequence that has 3, 2 or 1 amino acid difference with the CDR2 sequence mentioned in Table B-1 that belongs to the same combination; and a CDR3 sequence that has more than 80 % sequence identity with the CDR3 sequence mentioned in Table B-1 that belongs to the same combination; (2) a CDR1 sequence; a CDR 2 listed in Table B-1 and a CDR3 sequence listed in Table B-1 (in which the CDR2 sequence and CDR3 sequence may belong to different combinations).

Some even more preferred Nanobodies® of the invention may for example comprise: (1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table B-1; the CDR2 sequence listed in Table B-1 that belongs to the same combination; and a CDR3 sequence mentioned in Table B-1 that belongs to a different combination; or (2) a CDR1 sequence mentioned in Table B-1; a CDR2 sequence that has 3, 2 or 1 amino acid differences with the CDR2 sequence mentioned in Table B-1 that belongs to the same combination; and a CDR3 sequence that has more than 80% sequence identity with the CDR3 sequence listed in Table B-1 that belongs to the same or a different combination.

Particularly preferred Nanobodies® of the invention may for example comprise a CDR1 sequence mentioned in Table B-1, a CDR2 sequence that has more than 80 % sequence identity with

the CDR2 sequence mentioned in Table B-1 that belongs to the same combination; and the CDR3 sequence mentioned in Table B-1 that belongs to the same combination.

In the most preferred Nanobodies® of the invention, the CDR1, CDR2 and CDR3 sequences present are suitably chosen from one of the combinations of CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-1.

According to another preferred, but non-limiting aspect of the invention (a) CDR1 has a length of between 1 and 12 amino acid residues, and usually between 2 and 9 amino acid residues, such as 5, 6 or 7 amino acid residues; and/or (b) CDR2 has a length of between 13 and 24 amino acid residues, and usually between 15 and 21 amino acid residues, such as 16 and 17 amino acid residues; and/or (c) CDR3 has a length of between 2 and 35 amino acid residues, and usually between 3 and 30 amino acid residues, such as between 6 and 23 amino acid residues.

In another preferred, but non-limiting aspect, the invention relates to a Nanobody® in which the CDR sequences (as defined herein) have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1).

Generally, Nanobodies® with the above CDR sequences may be as further described herein, and preferably have framework sequences that are also as further described herein. Thus, for example and as mentioned herein, such Nanobodies® may be naturally occurring Nanobodies® (from any suitable species), naturally occurring V_{HH} sequences (i.e. from a suitable species of Camelid) or synthetic or semi-synthetic amino acid sequences or Nanobodies®, including but not limited to partially humanized Nanobodies® or V_{HH} sequences, fully humanized Nanobodies® or V_{HH} sequences, camelized heavy chain variable domain sequences, as well as Nanobodies® that have been obtained by the techniques mentioned herein.

Thus, in one specific, but non-limiting aspect, the invention relates to a humanized Nanobody®, which consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which CDR1 to CDR3 are as defined herein and in which said humanized Nanobody® comprises at least one humanizing substitution (as defined herein), and in particular at least one humanizing substitution in at least one of its framework sequences (as defined herein).

In another preferred, but non-limiting aspect, the invention relates to a Nanobody® in which the CDR sequences have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1). This degree of amino acid identity can for

example be determined by determining the degree of amino acid identity (in a manner described herein) between said Nanobody® and one or more of the sequences of SEQ ID NO's: 1589-1797 (see Table A-1), in which the amino acid residues that form the framework regions are disregarded. Such Nanobodies® can be as further described herein.

In another preferred, but non-limiting aspect, the invention relates to a Nanobody® with an amino acid sequence that is chosen from the group consisting of SEQ ID NO's: 1589-1797 (see Table A-1) or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1).

Another preferred, but non-limiting aspect of the invention relates to humanized variants of the Nanobodies® of SEQ ID NO's: 1589-1797 (see Table A-1), that comprise, compared to the corresponding native V_{HH} sequence, at least one humanizing substitution (as defined herein), and in particular at least one humanizing substitution in at least one of its framework sequences (as defined herein). Some preferred, but non-limiting examples of such humanized variants are the humanized Nanobodies® of SEQ ID NO's: 1798-1816 (see Table A-2). Thus, the invention also relates to a humanized Nanobody® with an amino acid sequence that is chosen from the group consisting of SEQ ID NO's: 1798-1816 (see Table A-2) or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with at least one of the amino acid sequences of SEQ ID NO's: 1798-1816 (see Table A-2) (in which amino acid sequences that are chosen from the latter group of amino acid sequences may contain a greater number or a smaller number of humanizing substitutions compared to the corresponding sequence of SEQ ID NO's: 1798-1816 (see Table A-2), as long as they retain at least one of the humanizing substitutions present in the corresponding sequence of SEQ ID NO's: 1798-1816 (see Table A-2)).

The polypeptides of the invention comprise or essentially consist of at least one Nanobody® of the invention. Some preferred, but non-limiting examples of polypeptides of the invention are given in SEQ ID NO's: 1817-1820 (see Table A-3).

It will be clear to the skilled person that the Nanobodies® that are mentioned herein as "preferred" (or "more preferred", "even more preferred", etc.) are also preferred (or more preferred, or even more preferred, etc.) for use in the polypeptides described herein. Thus, polypeptides that comprise or essentially consist of one or more "preferred" Nanobodies® of the invention will generally be preferred, and polypeptides that comprise or essentially consist of one or more "more preferred" Nanobodies® of the invention will generally be more preferred, etc.

Generally, proteins or polypeptides that comprise or essentially consist of a single Nanobody® (such as a single Nanobody® of the invention) will be referred to herein as "monovalent" proteins or polypeptides or as "monovalent constructs". Proteins and polypeptides that comprise or essentially consist of two or more Nanobodies® (such as at least two Nanobodies® of the invention or at least one Nanobody® of the invention and at least one other Nanobody®) will be referred to herein as "multivalent" proteins or polypeptides or as "multivalent constructs", and these may provide certain advantages compared to the corresponding monovalent Nanobodies® of the invention. Some non-limiting examples of such multivalent constructs will become clear from the further description herein.

According to one specific, but non-limiting aspect, a polypeptide of the invention comprises or essentially consists of at least two Nanobodies® of the invention, such as two or three Nanobodies® of the invention. As further described herein, such multivalent constructs can provide certain advantages compared to a protein or polypeptide comprising or essentially consisting of a single Nanobody® of the invention, such as a much improved avidity for Dkk-1. Such multivalent constructs will be clear to the skilled person based on the disclosure herein; some preferred, but non-limiting examples of such multivalent Nanobody® constructs are the constructs of SEQ ID NO's: 1817-1820.

According to another specific, but non-limiting aspect, a polypeptide of the invention comprises or essentially consists of at least one Nanobody® of the invention and at least one other binding unit (i.e. directed against another epitope, antigen, target, protein or polypeptide), which is preferably also a Nanobody®. Such proteins or polypeptides are also referred to herein as "multispecific" proteins or polypeptides or as 'multispecific constructs", and these may provide certain advantages compared to the corresponding monovalent Nanobodies® of the invention (as will become clear from the further discussion herein of some preferred, but-nonlimiting multispecific constructs). Such multispecific constructs will be clear to the skilled person based on the disclosure herein; some preferred, but non-limiting examples of such multispecific Nanobody® constructs are the constructs of SEQ ID NO's: 1817-1820.

According to yet another specific, but non-limiting aspect, a polypeptide of the invention comprises or essentially consists of at least one Nanobody® of the invention, optionally one or more further Nanobodies®, and at least one other amino acid sequence (such as a protein or polypeptide) that confers at least one desired property to the Nanobody® of the invention and/or to the resulting fusion protein. Again, such fusion proteins may provide certain advantages compared to the corresponding monovalent Nanobodies® of the invention. Some non-limiting examples of such

amino acid sequences and of such fusion constructs will become clear from the further description herein.

It is also possible to combine two or more of the above aspects, for example to provide a trivalent bispecific construct comprising two Nanobodies® of the invention and one other Nanobody®, and optionally one or more other amino acid sequences. Further non-limiting examples of such constructs, as well as some constructs that are particularly preferred within the context of the present invention, will become clear from the further description herein.

In the above constructs, the one or more Nanobodies® and/or other amino acid sequences may be directly linked to each other and/or suitably linked to each other via one or more linker sequences. Some suitable but non-limiting examples of such linkers will become clear from the further description herein.

In one specific aspect of the invention, a Nanobody® of the invention or a compound, construct or polypeptide of the invention comprising at least one Nanobody® of the invention may have an increased half-life, compared to the corresponding amino acid sequence of the invention. Some preferred, but non-limiting examples of such Nanobodies®, compounds and polypeptides will become clear to the skilled person based on the further disclosure herein, and for example comprise Nanobodies® sequences or polypeptides of the invention that have been chemically modified to increase the half-life thereof (for example, by means of pegylation); amino acid sequences of the invention that comprise at least one additional binding site for binding to a serum protein (such as serum albumin, see for example EP 0 368 684 B1, page 4); or polypeptides of the invention that comprise at least one Nanobody® of the invention that is linked to at least one moiety (and in particular at least one amino acid sequence) that increases the half-life of the Nanobody® of the invention. Examples of polypeptides of the invention that comprise such half-life extending moieties or amino acid sequences will become clear to the skilled person based on the further disclosure herein; and for example include, without limitation, polypeptides in which the one or more Nanobodies® of the invention are suitable linked to one or more serum proteins or fragments thereof (such as serum albumin or suitable fragments thereof) or to one or more binding units that can bind to serum proteins (such as, for example, Nanobodies® or (single) domain antibodies that can bind to serum proteins such as serum albumin, serum immunoglobulins such as IgG, or transferrine); polypeptides in which a Nanobody® of the invention is linked to an Fc portion (such as a human Fc) or a suitable part or fragment thereof; or polypeptides in which the one or more Nanobodies® of the invention are suitable linked to one or more small proteins or peptides that can bind to serum proteins (such as, without limitation, the proteins and peptides described in WO 91/01743, WO 01/45746, WO 02/076489 and to the US provisional application of Ablynx N.V.

entitled *"Peptides capable of binding to serum proteins"* of Ablynx N.V. filed on December 5, 2006 (see also PCT/EP/2007/063348).

Again, as will be clear to the skilled person, such Nanobodies®, compounds, constructs or polypeptides may contain one or more additional groups, residues, moieties or binding units, such as one or more further amino acid sequences and in particular one or more additional Nanobodies® (i.e. not directed against Dkk-1), so as to provide a tri- of multispecific Nanobody® construct.

Generally, the Nanobodies® of the invention (or compounds, constructs or polypeptides comprising the same) with increased half-life preferably have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding amino acid sequence of the invention per se. For example, the Nanobodies®, compounds, constructs or polypeptides of the invention with increased half-life may have a half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence of the invention per se.

In a preferred, but non-limiting aspect of the invention, such Nanobodies®, compound, constructs or polypeptides of the invention exhibit a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more. For example, compounds or polypeptides of the invention may have a half-life of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

In another one aspect of the invention, a polypeptide of the invention comprises one or more (such as two or preferably one) Nanobodies® of the invention linked (optionally via one or more suitable linker sequences) to one or more (such as two and preferably one) amino acid sequences that allow the resulting polypeptide of the invention to cross the blood brain barrier. In particular, said one or more amino acid sequences that allow the resulting polypeptides of the invention to cross the blood brain barrier may be one or more (such as two and preferably one) Nanobodies®, such as the Nanobodies® described in WO 02/057445, of which FC44 (SEQ ID NO: 189 of WO 06/040153) and FC5 (SEQ ID NO: 190 of WO 06/040154) are preferred examples.

In particular, polypeptides comprising one or more Nanobodies® of the invention are preferably such that they:

- bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e.

with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles);

and/or such that they:

- bind to Dkk-1 with a k_{on}-rate of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$;

and/or such that they:

- bind to Dkk-1 with a k_{off} rate between 1 s^{-1} ($t_{1/2}$=0.69 s) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Preferably, a polypeptide that contains only one amino acid sequence of the invention is preferably such that it will bind to Dkk-1 with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM. In this respect, it will be clear to the skilled person that a polypeptide that contains two or more Nanobodies® of the invention may bind to Dkk-1 with an increased avidity, compared to a polypeptide that contains only one amino acid sequence of the invention.

Some preferred IC_{50} values for binding of the amino acid sequences or polypeptides of the invention to Dkk-1 will become clear from the further description and examples herein.

Other polypeptides according to this preferred aspect of the invention may for example be chosen from the group consisting of amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more "sequence identity" (as defined herein) with one or more of the amino acid sequences of SEQ ID NO's: 1817-1820 (see Table A-3), in which the Nanobodies® comprised within said amino acid sequences are preferably as further defined herein.

Particularly preferred amino acid sequences of the invention (including but not limited to Nanobodies®) and polypeptides of the invention (including polypeptides that comprise one or more Nanobodies® of the invention) are preferably such that they bind to the LRP5/6 binding site on Dkk-1 (and in particular to one of the amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, preferably to one of the amino acid residues R242 or H210 of Dkk-1) and/or such that they can compete with LRP5/6 for binding to Dkk-1, and also:

- bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles);

and/or such that they:

- bind to Dkk-1 with a k_{on}-rate of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$;

and/or such that they:

- bind to Dkk-1 with a k_{off} rate between $1s^{-1}$ ($t_{1/2}$=0.69 s) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Other particularly preferred amino acid sequences of the invention (including but not limited to Nanobodies®) and polypeptides of the invention (including polypeptides that comprise one or more Nanobodies® of the invention) are preferably such that they bind to the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, preferably to at least one of amino acid residues R197 or K232 of Dkk-1) and/or such that they can compete with Kremen for binding to Dkk-1, and also:

- bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles);

and/or such that they:

- bind to Dkk-1 with a k_{on}-rate of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$;

and/or such that they:

- bind to Dkk-1 with a k_{off} rate between s^{-1} ($t_{1/2}$=0.69 s) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Some specifically particularly preferred polypeptides of the invention (including polypeptides that comprise one or more Nanobodies® of the invention) are preferably such that they (i) bind to the LRP5/6 binding site on Dkk-1 (and in particular to one of the amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, preferably to one of the amino acid residues R242 or H210 of Dkk-1) and/or can compete with LRP5/6 for binding to Dkk-1, and (ii) bind to the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, preferably to at least one of amino acid residues R197 or K232 of Dkk-1) and/or can compete with Kremen for binding to Dkk-1, and (iii) also:

- bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles);

and/or such that they:

- bind to Dkk-1 with a k_{on}-rate of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$;

and/or such that they:

- bind to Dkk-1 with a k_{off} rate between 1 s^{-1} ($t_{1/2}$=0.69 s) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Another aspect of this invention relates to a nucleic acid that encodes an amino acid sequence of the invention (such as a Nanobody® of the invention) or a polypeptide of the invention comprising the same. Again, as generally described herein for the nucleic acids of the invention, such a nucleic acid may be in the form of a genetic construct, as defined herein.

In another aspect, the invention relates to host or host cell that expresses or that is capable of expressing an amino acid sequence (such as a Nanobody®) of the invention and/or a polypeptide of the invention comprising the same; and/or that contains a nucleic acid of the invention. Some preferred but non-limiting examples of such hosts or host cells will become clear from the further description herein.

Another aspect of the invention relates to a product or composition containing or comprising at least one amino acid sequence of the invention, at least one polypeptide of the invention and/or at least one nucleic acid of the invention, and optionally one or more further components of such compositions known per se, i.e. depending on the intended use of the composition. Such a product or composition may for example be a pharmaceutical composition (as described herein), a veterinary composition or a product or composition for diagnostic use (as also described herein). Some preferred but non-limiting examples of such products or compositions will become clear from the further description herein.

The invention further relates to methods for preparing or generating the amino acid sequences, compounds, constructs, polypeptides, nucleic acids, host cells, products and compositions described herein. Some preferred but non-limiting examples of such methods will become clear from the further description herein.

The invention further relates to applications and uses of the amino acid sequences, compounds, constructs, polypeptides, nucleic acids, host cells, products and compositions described herein, as well as to methods for the prevention and/or treatment for diseases and disorders associated with Dkk-1. Some preferred but non-limiting applications and uses will become clear from the further description herein.

Other aspects, embodiments, advantages and applications of the invention will also become clear from the further description hereinbelow.

Generally, it should be noted that the term Nanobody® as used herein in its broadest sense is not limited to a specific biological source or to a specific method of preparation. For example, as will be discussed in more detail below, the Nanobodies® of the invention can generally be obtained by any of the techniques (1) to (8) mentioned on pages 61 and 62 of WO 08/020079, or any other suitable technique known per se. One preferred class of Nanobodies® corresponds to the V_{HH} domains of naturally occurring heavy chain antibodies directed against Dkk-1. As further described herein, such V_{HH} sequences can generally be generated or obtained by suitably immunizing a species of Camelid with Dkk-1 (i.e. so as to raise an immune response and/or heavy chain antibodies directed against Dkk-1), by obtaining a suitable biological sample from said Camelid (such as a blood sample, serum sample or sample of B-cells), and by generating V_{HH} sequences directed against Dkk-1, starting from said sample, using any suitable technique known per se. Such techniques will be clear to the skilled person and/or are further described herein.

Alternatively, such naturally occurring V_{HH} domains against Dkk-1, can be obtained from naïve libraries of Camelid V_{HH} sequences, for example by screening such a library using Dkk-1, or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known per se. Such libraries and techniques are for example described in WO 99/37681, WO 01/90190, WO 03/025020 and WO 03/035694. Alternatively, improved synthetic or semi-synthetic libraries derived from naïve V_{HH} libraries may be used, such as V_{HH} libraries obtained from naïve V_{HH} libraries by techniques such as random mutagenesis and/or CDR shuffling, as for example described in WO 00/43507.

Thus, in another aspect, the invention relates to a method for generating Nanobodies®, that are directed against Dkk-1. In one aspect, said method at least comprises the steps of:

a) providing a set, collection or library of Nanobody® sequences; and

b) screening said set, collection or library of Nanobody® sequences for Nanobody® sequences that can bind to and/or have affinity for Dkk-1;

and

c) isolating the Nanobody® or Nanobodies® that can bind to and/or have affinity for Dkk-1.

In such a method, the set, collection or library of Nanobody® sequences may be a naïve set, collection or library of Nanobody® sequences; a synthetic or semi-synthetic set, collection or library of Nanobody® sequences; and/or a set, collection or library of Nanobody® sequences that have been subjected to affinity maturation.

In a preferred aspect of this method, the set, collection or library of Nanobody® sequences may be an immune set, collection or library of Nanobody® sequences, and in particular an immune set, collection or library of V_{HH} sequences, that have been derived from a species of Camelid that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

In the above methods, the set, collection or library of Nanobody® or V_{HH} sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) Nanobody® sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to WO 03/054016 and to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

In another aspect, the method for generating Nanobody® sequences comprises at least the steps of:

a) providing a collection or sample of cells derived from a species of Camelid that express immunoglobulin sequences;

b) screening said collection or sample of cells for (i) cells that express an immunoglobulin sequence that can bind to and/or have affinity for Dkk-1; and (ii) cells that express heavy chain antibodies, in which substeps (i) and (ii) can be performed essentially as a single screening step or in any suitable order as two separate screening steps, so as to provide at least one cell that expresses a heavy chain antibody that can bind to and/or has affinity for Dkk-1;

and

c) either (i) isolating from said cell the V_{HH} sequence present in said heavy chain antibody; or (ii) isolating from said cell a nucleic acid sequence that encodes the V_{HH} sequence present in said heavy chain antibody, followed by expressing said V_{HH} domain.

In the method according to this aspect, the collection or sample of cells may for example be a collection or sample of B-cells. Also, in this method, the sample of cells may be derived from a Camelid that has been suitably immunized with Dkk-1 or a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other

epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The above method may be performed in any suitable manner, as will be clear to the skilled person. Reference is for example made to EP 0 542 810, WO 05/19824, WO 04/051268 and WO 04/106377. The screening of step b) is preferably performed using a flow cytometry technique such as FACS. For this, reference is for example made to Lieby et al., Blood, Vol. 97, No. 12, 3820. Particular reference is made to the so-called "Nanoclone™" technique described in international application WO 06/079372 by Ablynx N.V.

In another aspect, the method for generating an amino acid sequence directed against Dkk-1 may comprise at least the steps of:

a) providing a set, collection or library of nucleic acid sequences encoding heavy chain antibodies or Nanobody® sequences;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode a heavy chain antibody or a Nanobody® sequence that can bind to and/or has affinity for Dkk-1;

and

c) isolating said nucleic acid sequence, followed by expressing the V_{HH} sequence present in said heavy chain antibody or by expressing said Nanobody® sequence, respectively.

In such a method, the set, collection or library of nucleic acid sequences encoding heavy chain antibodies or Nanobody® sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of heavy chain antibodies or V_{HH} sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of Nanobody® sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of Nanobody® sequences that have been subjected to affinity maturation.

In a preferred aspect of this method, the set, collection or library of nucleic acid sequences may be an immune set, collection or library of nucleic acid sequences encoding heavy chain antibodies or V_{HH} sequences derived from a Camelid that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

In the above methods, the set, collection or library of nucleotide sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a

set, collection or library of) nucleotide sequences encoding amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to WO 03/054016 and to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

Also encompassed within the present invention are methods for preparing and generating multiparatopic (such as e.g. biparatopic, triparatopic, etc.) amino acids of the invention.

Without being limiting, a method for preparing and generating biparatopic amino acids of the invention may comprise at least the steps of:

a) providing a nucleic acid sequence encoding a Dkk-1 binding amino acid sequence fused to a set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for an antigenic determinant on Dkk-1 different from the antigenic determinant recognized by the Dkk-1 binding amino acid sequence;

and

c) isolating the nucleic acid sequence encoding the Dkk-1 binding amino acid sequence fused to the nucleic acid sequence obtained in b), followed by expressing the encoded amino acid sequence.

The biparatopic amino acid sequence obtained in the method above, can subsequently be fused to one or more further sets, collections or libraries of nucleic acid sequences encoding amino acid sequences and again screened for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for an antigenic determinant on Dkk-1 different from the antigenic determinant of the Dkk-1 binding amino acid sequence and the antigenic determinant of b) in order to obtain a triparatopic or multiparatopic amino acid sequence respectively.

In such a method, the set, collection or library of nucleic acid sequences encoding amino acid sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

Also, in such a method, the set, collection or library of nucleic acid sequences may encode a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of nucleic acid sequences may encode a set, collection or library of domain antibodies or single domain antibodies, or a set,

collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of amino acid sequences may be an immune set, collection or library of nucleic acid sequences, for example derived from a mammal that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The set, collection or library of nucleic acid sequences may for example encode an immune set, collection or library of heavy chain variable domains or of light chain variable domains. In one specific aspect, the set, collection or library of nucleotide sequences may encode a set, collection or library of V_{HH} sequences.

In the above methods, the nucleic acid sequence encoding a Dkk-1 binding amino acid sequence fused to the set, collection or library of nucleotide sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) nucleotide sequences encoding amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

According to a particularly preferred aspect, a method for preparing and generating biparatopic amino acids of the invention may comprise at least the steps of:

a) providing a set, collection or library of nucleic acid sequences, in which each nucleic acid sequence in said set, collection or library encodes a fusion protein that comprises a first amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1 that is fused (optionally via a linker sequence) to a second amino acid sequence, in which essentially each second amino acid sequence (or most of these) is a different member of a set, collection or library of different amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on Dkk-1 different from the first antigenic determinant, part, domain or epitope on Dkk-1;

and

c) isolating the nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on Dkk-1

different from the first antigenic determinant, part, domain or epitope on Dkk-1, obtained in b), optionally followed by expressing the encoded amino acid sequence.

In this preferred method, the first amino acid sequence in the fusion protein encoded by said set collection or library of nucleic acid sequences may be the same amino acid sequence for all members of the set, collection or library of nucleic acid sequences encoding the fusion protein; or the first amino acid sequence in the fusion protein encoded by said set collection or library of nucleic acid sequences may also be a member of a set collection or library of different amino acid sequences.

Again, in such a method, the set, collection or library of nucleic acid sequences encoding amino acid sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

Also, in such a method, the set, collection or library of nucleic acid sequences may encode a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of nucleic acid sequences may encode a set, collection or library of domain antibodies or single domain antibodies, or a set, collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of amino acid sequences may be an immune set, collection or library of nucleic acid sequences, for example derived from a mammal that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The set, collection or library of nucleic acid sequences may for example encode an immune set, collection or library of heavy chain variable domains or of light chain variable domains. In one specific aspect, the set, collection or library of nucleotide sequences may encode a set, collection or library of V_{HH} sequences.

In the above methods, the nucleic acid sequence encoding a Dkk-1 binding amino acid sequence fused to the set, collection or library of nucleotide sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate

screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) nucleotide sequences encoding amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

In step b), the set, collection or library of nucleic acid sequences may also be screened for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for both the first antigenic determinant, part, domain or epitope on Dkk-1 and the second antigenic determinant, part, domain or epitope on Dkk-1. This may for example be performed in a subsequent steps (i.e. by in a first step screening or selecting for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the second antigenic determinant, part, domain or epitope on Dkk-1, and subsequently in a second step selecting or screening for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the first antigenic determinant, part, domain or epitope on Dkk-1; or visa versa) or in a single step (i.e. by simultaneously screening or selecting for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for both the first antigenic determinant, part, domain or epitope on Dkk-1 and the second antigenic determinant, part, domain or epitope on Dkk-1).

In a preferred aspect of the above method, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 on Dkk-1, preferably to at least one of amino acid residues R242 or H210 on Dkk-1) and/or (ii) competes with LRP5/6 for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) an amino acid sequence that can compete with Kremen for binding to Dkk-1.

Alternatively, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular to at least one of amino acid residues W212, R242, K217, R209, H210, L266,

or H267 on Dkk-1, preferably to at least one of amino acid residues R242 or H210 on Dkk-1) and/or (ii) an amino acid sequence that can compete with LRP5/6 for binding to Dkk-1.

In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with LRP5/6 or Kremen, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

It is also possible, in step b), to screen for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1; and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 on Dkk-1, preferably to at least one of amino acid residues R242 or H210 on Dkk-1) and/or that can compete with LRP5/6 for binding to Dkk-1. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of LRP5/6 and/or Kremen, as applicable.

In another preferred aspect of the above method, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or (ii) it competes with BHQ880 for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) an amino acid sequence that can compete with Kremen for binding to Dkk-1.

Alternatively, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) it competes with Kremen for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or (ii) an amino acid sequence that can compete with BHQ880 for binding to Dkk-1.

In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with BHQ880 or Kremen, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

It is also possible, in step b), to screen for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1; and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or that can compete with BHQ880 for binding to Dkk-1. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of BHQ880 and/or Kremen, as applicable.

In another preferred aspect of the above method, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or (ii) it competes with RH2-18 for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) an amino acid sequence that can compete with Kremen for binding to Dkk-1.

Alternatively, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) it competes with Kremen for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or (ii) an amino acid sequence that can compete with RH2-18 for binding to Dkk-1.

In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with RH2-18 or Kremen, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding

molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

It is also possible, in step b), to screen for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1; and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or that can compete with RH2-18 for binding to Dkk-1. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of RH2-18 and/or Kremen, as applicable.

In another preferred aspect of the above method, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or (ii) it competes with 11H10 for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) an amino acid sequence that can compete with Kremen for binding to Dkk-1.

Alternatively, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) it competes with Kremen for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or (ii) an amino acid sequence that can compete with 11H10 for binding to Dkk-1.

In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with 11H10 or Kremen, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

It is also possible, in step b), to screen for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to

at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1; and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or that can compete with 11H10 for binding to Dkk-1. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of BHQ880 and/or Kremen, as applicable.

It will also be clear to the skilled person that the above methods may be performed by screening a set, collection or library of amino acid sequences that correspond to (e.g. are encoded by) the nucleic acid sequences used in the above method; and such methods form further aspects of the invention.

The invention in a further aspect provides a method for preparing and generating biparatopic amino acids of the invention which comprises at least the steps of:

a) providing a set, collection or library of nucleic acid sequences, in which each nucleic acid sequence in said set, collection or library encodes a fusion protein that comprises a first amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1 that is fused via a linker sequence to a second amino acid sequence that has can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on Dkk-1 (which may be the same or different as the first antigenic determinant, part, domain or epitope on Dkk-1), in which essentially each nucleic acid sequence (or most of these) encodes a fusion protein with a different linker sequence so as to provide a set, collection or library encoding different fusion proteins;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on Dkk-1;

and

c) isolating the nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on Dkk-1, optionally followed by expressing the encoded amino acid sequence.

As will be clear to the skilled person, this method can be used to screen for suitable or even optimal linker lengths for linking the first and second amino acid sequence. For example, in this aspect, the first amino acid sequence may be an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular for at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 on Dkk-1, preferably for at least one of amino acid residues R242 or H210 on Dkk-1) and/or that can compete with LRP5/6 for binding to Dkk-1; and the second amino acid sequence may be an amino acid sequence that can bind to and/or has affinity for

the Kremen binding site on Dkk-1 (and in particular for at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably for at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1 (or visa versa). The screening and selection step b) may be performed as further described above.

For example, in this aspect, the first amino acid sequence may be an amino acid sequence that can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or that can compete with BHQ880 for binding to Dkk-1; and the second amino acid sequence may be an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular for at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably for at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1 (or visa versa). The screening and selection step b) may be performed as further described above.

For example, in this aspect, the first amino acid sequence may be an amino acid sequence that can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or that can compete with RH2-18 for binding to Dkk-1; and the second amino acid sequence may be an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular for at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably for at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1 (or visa versa). The screening and selection step b) may be performed as further described above.

For example, in this aspect, the first amino acid sequence may be an amino acid sequence that can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or that can compete with 11H10 for binding to Dkk-1; and the second amino acid sequence may be an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular for at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably for at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1 (or visa versa). The screening and selection step b) may be performed as further described above.

Another method for preparing and generating biparatopic amino acids of the invention may comprise at least the steps of:

a) providing a set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for a set, collection or library of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1;

c) ligating said set, collection or library of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 to another nucleic acid sequence that encodes an amino acid sequence that can bind to and/or has affinity for Dkk-1 (e.g. a nucleic acid sequence that encodes an amino acid sequence that competes with LRP5/6 for binding Dkk-1);

and

d) from the set, collection or library of nucleic acid sequences obtained in c), isolating the nucleic acid sequences encoding a biparatopic amino acid sequence that can bind to and/or has affinity for Dkk-1 (and e.g. further selecting for nucleic acid sequences that encode a biparatopic amino acid sequence that antagonizes with higher potency compared to the monovalent amino acid sequences), followed by expressing the encoded amino acid sequence.

The biparatopic amino acid sequence obtained in the method above, can subsequently be fused to one or more further sets, collections or libraries of nucleic acid sequences encoding amino acid sequences that can bind to and/or have affinity for Dkk-1 in order to obtain a triparatopic or multiparatopic amino acid sequence respectively.

In such a method, the set, collection or library of nucleic acid sequences encoding amino acid sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

The set, collection or library of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 can be obtained by any selection or screening method known in the art for the selection and/or screening of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 and as, for example, described in the Examples section.

Also, in such a method, the set, collection or library of nucleic acid sequences may encode a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of nucleic acid sequences may encode a set, collection or library of domain antibodies or single domain antibodies, or a set,

collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of amino acid sequences may be an immune set, collection or library of nucleic acid sequences, for example derived from a mammal that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The set, collection or library of nucleic acid sequences may for example encode an immune set, collection or library of heavy chain variable domains or of light chain variable domains. In one specific aspect, the set, collection or library of nucleotide sequences may encode a set, collection or library of V_{HH} sequences.

In the above methods, the nucleic acid sequence may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) nucleotide sequences encoding amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disciosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

Another method for preparing and generating biparatopic amino acids of the invention may comprise at least the steps of:

a) providing a first set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said first set, collection or library of nucleic acid sequences for a nucleic acid sequence that encodes an amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1;

c) ligating the nucleic acid sequence encoding said amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1 to another set, collection or library of nucleic acid sequences encoding amino acid sequences to obtain a set, collection or library of nucleic acid sequences that encode fusion proteins;

d) screening said set, collection or library of nucleic acid sequences obtained in step c) for a nucleic acid sequence that encodes an amino acid sequence that can bind a second antigenic determinant, part, domain or epitope on Dkk-1 different from the first antigenic determinant, part, domain or epitope on Dkk-1;

and

e) isolating the nucleic acid sequence that encodes an amino acid sequence that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on Dkk-1, optionally followed by expressing the encoded amino acid sequence.

In a preferred aspect of the above method, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 on Dkk-1, preferably to at least one of amino acid residues R242 or H210 on Dkk-1) and/or (ii) competes with LRP5/6 for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) an amino acid sequence that can compete with Kremen for binding to Dkk-1.

Alternatively, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 on Dkk-1, preferably to at least one of amino acid residues R242 or H210 on Dkk-1) and/or (ii) an amino acid sequence that can compete with LRP5/6 for binding to Dkk-1.

In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with LRP5/6 or Kremen, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

It is also possible, in step b), to screen for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1; and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular to at least one of amino acid residues

W212, R242, K217, R209, H210, L266, or H267 on Dkk-1, preferably to at least one of amino acid residues R242 or H210 on Dkk-1) and/or that can compete with LRP5/6 for binding to Dkk-1. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of LRP5/6 and/or Kremen, as applicable.

In a preferred aspect of the above method, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or (ii) it competes with BHQ880 for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) an amino acid sequence that can compete with Kremen for binding to Dkk-1.

Alternatively, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or (ii) an amino acid sequence that can compete with BHQ880for binding to Dkk-1.

In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with BHQ880 or Kremen, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

It is also possible, in step b), to screen for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1; and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or that can compete with BHQ880 for binding to Dkk-1. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of BHQ880 and/or Kremen, as applicable.

In another preferred aspect of the above method, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or (ii) it competes with 11H10 for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) an amino acid sequence that can compete with Kremen for binding to Dkk-1.

Alternatively, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or (ii) an amino acid sequence that can compete with 11H10 for binding to Dkk-1.

In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with 11H10 or Kremen, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

It is also possible, in step b), to screen for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1; and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or that can compete with 11H10 for binding to Dkk-1. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of 11H10 and/or Kremen, as applicable.

In another preferred aspect of the above method, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or (ii) it competes with RH2-18 for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular

to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) an amino acid sequence that can compete with Kremen for binding to Dkk-1.

Alternatively, the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1; and in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or (ii) an amino acid sequence that can compete with RH2-18 for binding to Dkk-1.

In the above methods, screening or selecting for (nucleic acid sequences that encode) amino acid sequences that compete with RH2-18 or Kremen, respectively, may be performed using generally known methods for screening or selecting for competitors of known binding molecules, which may for example involve performing the screening or selection in the presence of the binding molecule and/or determining the binding affinity of the compound(s) to be screened in the presence of the binding molecule.

It is also possible, in step b), to screen for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1) and/or that can compete with Kremen for binding to Dkk-1; and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or that can compete with RH2-18 for binding to Dkk-1. Again, this may be performed in separate steps or a single step, and by selecting or screening in the presence of RH2-18 and/or Kremen, as applicable.

The biparatopic amino acid sequence obtained in the method above, can subsequently be fused to one or more further sets, collections or libraries of nucleic acid sequences encoding amino acid sequences that can bind to and/or have affinity for Dkk-1 in order to obtain a triparatopic or multiparatopic amino acid sequence respectively.

In such a method, the set, collection or library of nucleic acid sequences encoding amino acid sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

The set, collection or library of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 can be obtained by any selection or screening method known in the art for the selection and/or screening of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 and as, for example, described in the Examples section.

Also, in such a method, the set, collection or library of nucleic acid sequences may encode a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of nucleic acid sequences may encode a set, collection or library of domain antibodies or single domain antibodies, or a set, collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of amino acid sequences may be an immune set, collection or library of nucleic acid sequences, for example derived from a mammal that has been suitably immunized with Dkk-1 or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The set, collection or library of nucleic acid sequences may for example encode an immune set, collection or library of heavy chain variable domains or of light chain variable domains. In one specific aspect, the set, collection or library of nucleotide sequences may encode a set, collection or library of V_{HH} sequences.

In the above methods, the nucleic acid sequence may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) nucleotide sequences encoding amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

As will be clear to the skilled person, the screening step of the methods described herein can also be performed as a selection step. Accordingly the term "screening" as used in the present description can comprise selection, screening or any suitable combination of selection and/or screening techniques. Also, when a set, collection or library of sequences is used, it may contain any suitable number of sequences, such as 1, 2, 3 or about 5, 10, 50, 100, 500, 1000, 5000, 10^4, 10^5, 10^6, 10^7, 10^8 or more sequences.

Also, one or more or all of the sequences in the above set, collection or library of amino acid sequences may be obtained or defined by rational, or semi-empirical approaches such as computer modelling techniques or biostatics or datamining techniques.

Furthermore, such a set, collection or library can comprise one, two or more sequences that are variants from one another (e.g. with designed point mutations or with randomized positions), compromise multiple sequences derived from a diverse set of naturally diversified sequences (e.g. an immune library)), or any other source of diverse sequences (as described for example in Hoogenboom et al, Nat Biotechnol 23:1105, 2005 and Binz et al, Nat Biotechnol 2005, 23:1247). Such set, collection or library of sequences can be displayed on the surface of a phage particle, a ribosome, a bacterium, a yeast cell, a mammalian cell, and linked to the nucleotide sequence encoding the amino acid sequence within these carriers. This makes such set, collection or library amenable to selection procedures to isolate the desired amino acid sequences of the invention. More generally, when a sequence is displayed on a suitable host or host cell, it is also possible (and customary) to first isolate from said host or host cell a nucleotide sequence that encodes the desired sequence, and then to obtain the desired sequence by suitably expressing said nucleotide sequence in a suitable host organism. Again, this can be performed in any suitable manner known per se, as will be clear to the skilled person.

Yet another technique for obtaining V_{HH} sequences or Nanobody® sequences directed against Dkk-1 involves suitably immunizing a transgenic mammal that is capable of expressing heavy chain antibodies (i.e. so as to raise an immune response and/or heavy chain antibodies directed against Dkk-1), obtaining a suitable biological sample from said transgenic mammal that contains (nucleic acid sequences encoding) said V_{HH} sequences or Nanobody® sequences (such as a blood sample, serum sample or sample of B-cells), and then generating V_{HH} sequences directed against Dkk-1, starting from said sample, using any suitable technique known per se (such as any of the methods described herein or a hybridoma technique). For example, for this purpose, the heavy chain antibody-expressing mice and the further methods and techniques described in WO 02/085945, WO 04/049794 and WO 06/008548 and Janssens et al., Proc. Natl. Acad. Sci .USA. 2006 Oct 10;103(41):15130-5 can be used. For example, such heavy chain antibody expressing mice can express heavy chain antibodies with any suitable (single) variable domain, such as (single) variable domains from natural sources (e.g. human (single) variable domains, Camelid (single) variable domains or shark (single) variable domains), as well as for example synthetic or semi-synthetic (single) variable domains.

The invention also relates to the V_{HH} sequences or Nanobody® sequences that are obtained by the above methods, or alternatively by a method that comprises the one of the above methods and

in addition at least the steps of determining the nucleotide sequence or amino acid sequence of said V_{HH} sequence or Nanobody® sequence; and of expressing or synthesizing said V_{HH} sequence or Nanobody® sequence in a manner known per se, such as by expression in a suitable host cell or host organism or by chemical synthesis.

As mentioned herein, a particularly preferred class of Nanobodies® of the invention comprises Nanobodies® with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring V_{HH} domain, but that has been "humanized", i.e. by replacing one or more amino acid residues in the amino acid sequence of said naturally occurring V_{HH} sequence (and in particular in the framework sequences) by one or more of the amino acid residues that occur at the corresponding position(s) in a V_H domain from a conventional 4-chain antibody from a human being (e.g. indicated above), as further described on, and using the techniques mentioned on, page 63 of WO 08/020079. Another particularly preferred class of Nanobodies® of the invention comprises Nanobodies® with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring V_H domain, but that has been "camelized", i.e. by replacing one or more amino acid residues in the amino acid sequence of a naturally occurring V_H domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a V_{HH} domain of a heavy chain antibody, as further described on, and using the techniques mentioned on, page 63 of WO 08/020079.

Other suitable methods and techniques for obtaining the Nanobodies® of the invention and/or nucleic acids encoding the same, starting from naturally occurring V_H sequences or preferably V_{HH} sequences, will be clear from the skilled person, and may for example include the techniques that are mentioned on page 64 of WO 08/00279As mentioned herein, Nanobodies® may in particular be characterized by the presence of one or more "*Hallmark residues*" (as described herein) in one or more of the framework sequences.

Thus, according to one preferred, but non-limiting aspect of the invention, a Nanobody® in its broadest sense can be generally defined as a polypeptide comprising:

a) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 108 according to the Kabat numbering is Q;

and/or:

b) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 45 according to the Kabat numbering is a charged amino acid (as defined herein) or a cysteine residue, and position 44 is preferably an E;

and/or:

c) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S.

Thus, in a first preferred, but non-limiting aspect, a Nanobody® of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which

a) the amino acid residue at position 108 according to the Kabat numbering is Q;

and/or in which:

b) the amino acid residue at position 45 according to the Kabat numbering is a charged amino acid or a cysteine and the amino acid residue at position 44 according to the Kabat numbering is preferably E;

and/or in which:

c) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S;

and in which:

d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

In particular, a Nanobody® in its broadest sense can be generally defined as a polypeptide comprising:

a) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 108 according to the Kabat numbering is Q;

and/or:

b) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 44 according to the Kabat numbering is E and in which the amino acid residue at position 45 according to the Kabat numbering is an R;

and/or:

c) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S.

Thus, according to a preferred, but non-limiting aspect, a Nanobody® of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which

a) the amino acid residue at position 108 according to the Kabat numbering is Q;

and/or in which:

b) the amino acid residue at position 44 according to the Kabat numbering is E and in which the amino acid residue at position 45 according to the Kabat numbering is an R;

and/or in which:

c) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S;

and in which:

d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

In particular, a Nanobody® against Dkk-1 according to the invention may have the structure:

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which

a) the amino acid residue at position 108 according to the Kabat numbering is Q;

and/or in which:

b) the amino acid residue at position 44 according to the Kabat numbering is E and in which the amino acid residue at position 45 according to the Kabat numbering is an R;

and/or in which:

c) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S;

and in which:

d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

In particular, according to one preferred, but non-limiting aspect of the invention, a Nanobody® can generally be defined as a polypeptide comprising an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which;

a-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of A, G, E, D, G, Q, R, S, L; and is preferably chosen from the group consisting of G, E or Q; and

a-2) the amino acid residue at position 45 according to the Kabat numbering is chosen from the group consisting of L, R or C; and is preferably chosen from the group consisting of L or R; and

a-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of W, R or S; and is preferably W or R, and is most preferably W;

a-4) the amino acid residue at position 108 according to the Kabat numbering is Q;

or in which:

b-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of E and Q; and

b-2) the amino acid residue at position 45 according to the Kabat numbering is R; and

b-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of W, R and S; and is preferably W;

b-4) the amino acid residue at position 108 according to the Kabat numbering is chosen from the group consisting of Q and L; and is preferably Q;

or in which:

c-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of A, G, E, D, Q, R, S and L; and is preferably chosen from the group consisting of G, E and Q; and

c-2) the amino acid residue at position 45 according to the Kabat numbering is chosen from the group consisting of L, R and C; and is preferably chosen from the group consisting of L and R; and

c-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S; and is in particular chosen from the group consisting of R and S; and

c-4) the amino acid residue at position 108 according to the Kabat numbering is chosen from the group consisting of Q and L; is preferably Q;

and in which

d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

Thus, in another preferred, but non-limiting aspect, a Nanobody® of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

a-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of A, G, E, D, G, Q, R, S, L; and is preferably chosen from the group consisting of G, E or Q;

and in which:

a-2) the amino acid residue at position 45 according to the Kabat numbering is chosen from the group consisting of L, R or C; and is preferably chosen from the group consisting of L or R;

and in which:

a-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of W, R or S; and is preferably W or R, and is most preferably W;

and in which

a-4) the amino acid residue at position 108 according to the Kabat numbering is Q;

and in which:

d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

In another preferred, but non-limiting aspect, a Nanobody® of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

b-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of E and Q;

and in which:

b-2) the amino acid residue at position 45 according to the Kabat numbering is R;

and in which:

b-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of W, R and S; and is preferably W;

and in which:

b-4) the amino acid residue at position 108 according to the Kabat numbering is chosen from the group consisting of Q and L; and is preferably Q;

and in which:

d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

In another preferred, but non-limiting aspect, a Nanobody® of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

c-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of A, G, E, D, Q, R, S and L; and is preferably chosen from the group consisting of G, E and Q;

and in which:

c-2) the amino acid residue at position 45 according to the Kabat numbering is chosen from the group consisting of L, R and C; and is preferably chosen from the group consisting of L and R;

and in which:

c-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S; and is in particular chosen from the group consisting of R and S;

and in which:

c-4) the amino acid residue at position 108 according to the Kabat numbering is chosen from the group consisting of Q and L; is preferably Q;

and in which:

d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

Two particularly preferred, but non-limiting groups of the Nanobodies® of the invention are those according to a) above; according to (a-1) to (a-4) above; according to b) above; according to (b-1) to (b-4) above; according to (c) above; and/or according to (c-1) to (c-4) above, in which either:

i) the amino acid residues at positions 44-47 according to the Kabat numbering form the sequence GLEW (or a GLEW-like sequence as described herein) and the amino acid residue at position 108 is Q;

or in which:

ii) the amino acid residues at positions 43-46 according to the Kabat numbering form the sequence KERE or KQRE (or a KERE-like sequence as described) and the amino acid residue at position 108 is Q or L, and is preferably Q.

Thus, in another preferred, but non-limiting aspect, a Nanobody® of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) the amino acid residues at positions 44-47 according to the Kabat numbering form the sequence GLEW (or a GLEW-like sequence as defined herein) and the amino acid residue at position 108 is Q;

and in which:

ii) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

In another preferred, but non-limiting aspect, a Nanobody® of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) the amino acid residues at positions 43-46 according to the Kabat numbering form the sequence KERE or KQRE (or a KERE-like sequence) and the amino acid residue at position 108 is Q or L, and is preferably Q;

and in which:

ii) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

In the Nanobodies® of the invention in which the amino acid residues at positions 43-46 according to the Kabat numbering form the sequence KERE or KQRE, the amino acid residue at position 37 is most preferably F. In the Nanobodies® of the invention in which the amino acid residues at positions 44-47 according to the Kabat numbering form the sequence GLEW, the amino acid residue at position 37 is chosen from the group consisting of Y, H, I, L, V or F, and is most preferably V.

Thus, without being limited hereto in any way, on the basis of the amino acid residues present on the positions mentioned above, the Nanobodies® of the invention can generally be classified on the basis of the following three groups:

i) The "*GLEW-group*": Nanobodies® with the amino acid sequence GLEW at positions 44-47 according to the Kabat numbering and Q at position 108 according to the Kabat numbering. As further described herein, Nanobodies® within this group usually have a V at position 37, and can have a W, P, R or S at position 103, and preferably have a W at position 103. The GLEW group also comprises some GLEW-like sequences such as those mentioned in Table B-2 below. More generally, and without limitation, Nanobodies® belonging to the GLEW-group can be defined as Nanobodies® with a G at position 44 and/or with a W at position 47, in which position 46 is usually E and in which preferably position 45 is not a charged amino acid residue and not cysteine;

ii) The "*KERE-group*": Nanobodies® with the amino acid sequence KERE or KQRE (or another KERE-like sequence) at positions 43-46 according to the Kabat numbering and Q or L at position 108 according to the Kabat numbering. As further described herein, Nanobodies® within this group usually have a F at position 37, an L or F at position 47; and can have a W, P, R or S at position 103, and preferably have a W at position 103. More generally, and without

limitation, Nanobodies® belonging to the KERE-group can be defined as Nanobodies® with a K, Q or R at position 44 (usually K) in which position 45 is a charged amino acid residue or cysteine, and position 47 is as further defined herein;

iii) The "*103 P, R, S-group*": Nanobodies® with a P, R or S at position 103. These Nanobodies® can have either the amino acid sequence GLEW at positions 44-47 according to the Kabat numbering or the amino acid sequence KERE or KQRE at positions 43-46 according to the Kabat numbering, the latter most preferably in combination with an F at position 37 and an L or an F at position 47 (as defined for the KERE-group); and can have Q or L at position 108 according to the Kabat numbering, and preferably have Q.

Also, where appropriate, Nanobodies® may belong to (i.e. have characteristics of) two or more of these classes. For example, one specifically preferred group of Nanobodies® has GLEW or a GLEW-like sequence at positions 44-47; P,R or S (and in particular R) at position 103; and Q at position 108 (which may be humanized to L).

More generally, it should be noted that the definitions referred to above describe and apply to Nanobodies® in the form of a native (i.e. non-humanized) V_{HH} sequence, and that humanized variants of these Nanobodies® may contain other amino acid residues than those indicated above (i.e. one or more humanizing substitutions as defined herein). For example, and without limitation, in some humanized Nanobodies® of the GLEW-group or the 103 P, R, S-group, Q at position 108 may be humanized to 108L. As already mentioned herein, other humanizing substitutions (and suitable combinations thereof) will become clear to the skilled person based on the disclosure herein. In addition, or alternatively, other potentially useful humanizing substitutions can be ascertained by comparing the sequence of the framework regions of a naturally occurring V_{HH} sequence with the corresponding framework sequence of one or more closely related human V_H sequences, after which one or more of the potentially useful humanizing substitutions (or combinations thereof) thus determined can be introduced into said V_{HH} sequence (in any manner known per se, as further described herein) and the resulting humanized V_{HH} sequences can be tested for affinity for the target, for stability, for ease and level of expression, and/or for other desired properties. In this way, by means of a limited degree of trial and error, other suitable humanizing substitutions (or suitable combinations thereof) can be determined by the skilled person based on the disclosure herein. Also, based on the foregoing, (the framework regions of) a Nanobody® may be partially humanized or fully humanized.

Thus, in another preferred, but non-limiting aspect, a Nanobody® of the invention may be a Nanobody® belonging to the GLEW-group (as defined herein), and in which CDR1, CDR2 and CDR3

are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

In another preferred, but non-limiting aspect, a Nanobody® of the invention may be a Nanobody® belonging to the KERE-group (as defined herein), and CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

Thus, in another preferred, but non-limiting aspect, a Nanobody® of the invention may be a Nanobody® belonging to the 103 P, R, S-group (as defined herein), and in which CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

Also, more generally and in addition to the 108Q, 43E/44R and 103 P,R,S residues mentioned above, the Nanobodies® of the invention can contain, at one or more positions that in a conventional V_H domain would form (part of) the V_H/V_L interface, one or more amino acid residues that are more highly charged than the amino acid residues that naturally occur at the same position(s) in the corresponding naturally occurring V_H sequence, and in particular one or more charged amino acid residues (as mentioned in Table A-2 on page 48 of the International application WO 08/020079). Such substitutions include, but are not limited to, the GLEW-like sequences mentioned in Table B-2 below; as well as the substitutions that are described in the International Application WO 00/29004 for so-called "microbodies", e.g. so as to obtain a Nanobody® with Q at position 108 in combination with KLEW at positions 44-47. Other possible substitutions at these positions will be clear to the skilled person based upon the disclosure herein.

In one aspect of the Nanobodies® of the invention, the amino acid residue at position 83 is chosen from the group consisting of L, M, S, V and W; and is preferably L.

Also, in one aspect of the Nanobodies® of the invention, the amino acid residue at position 83 is chosen from the group consisting of R, K, N, E, G, I, T and Q; and is most preferably either K or E (for Nanobodies® corresponding to naturally occurring V_{HH} domains) or R (for "humanized" Nanobodies®, as described herein). The amino acid residue at position 84 is chosen from the group consisting of P, A, R, S, D T, and V in one aspect, and is most preferably P (for Nanobodies® corresponding to naturally occurring V_{HH} domains) or R (for "humanized" Nanobodies®, as described herein).

Furthermore, in one aspect of the Nanobodies® of the invention, the amino acid residue at position 104 is chosen from the group consisting of G and D; and is most preferably G.

Collectively, the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108, which in the Nanobodies® are as mentioned above, will also be referred to herein as the "Hallmark

Residues". The Hallmark Residues and the amino acid residues at the corresponding positions of the most closely related human V_H domain, V_H3, are summarized in Table B-2.

Some especially preferred but non-limiting combinations of these Hallmark Residues as occur in naturally occurring V_{HH} domains are mentioned in Table B-3. For comparison, the corresponding amino acid residues of the human V_H3 called DP-47 have been indicated in italics.

Table B-2: Hallmark Residues in Nanobodies®

Position	Human V_H3	Hallmark Residues
11	L, V; predominantly L	L, S, V, M, W, F, T, Q, E, A, R, G, K, Y, N, P, I; preferably L
37	V, I, F; usually V	$F^{(1)}$, Y, V, L, A, H, S, I, W, C, N, G, D, T, P, preferably $F^{(1)}$ or Y
$44^{(8)}$	G	$E^{(3)}$, $Q^{(3)}$, $G^{(2)}$, D, A, K, R, L, P, S, V, H, T, N, W, M, I; preferably $G^{(2)}$, $E^{(3)}$ or $Q^{(3)}$;most preferably $G^{(2)}$ or $Q^{(3)}$.
$45^{(8)}$	L	$L^{(2)}$, $R^{(3)}$, P, H, F, G, Q, S, E, T, Y, C, I, D, V; preferably $L^{(2)}$ or $R^{(3)}$
$47^{(8)}$	W, Y	$F^{(1)}$, $L^{(1)}$ or $W^{(2)}$ G, I, S, A, V, M, R, Y, E, P, T, C, H, K, Q, N, D; preferably $W^{(2)}$, $L^{(1)}$ or $F^{(1)}$
83	R or K; usually R	R, $K^{(5)}$, T, $E^{(5)}$, Q, N, S, I, V, G, M, L, A, D, Y, H; preferably K or R; most preferably K
84	A, T, D; predominantly A	$P^{(5)}$, S, H, L, A, V, I, T, F, D, R, Y, N, Q, G, E; preferably P
103	W	$W^{(4)}$, $R^{(6)}$, G, S, K, A, M, Y, L, F, T, N, V, Q, $P^{(6)}$, E, C; preferably W
104	G	G, A, S, T, D, P, N, E, C, L; preferably G
108	L, M or T; predominantly L	Q, $L^{(7)}$, R, P, E, K, S, T, M, A, H; preferably Q or $L^{(7)}$

Notes:

(1) In particular, but not exclusively, in combination with KERE or KQRE at positions 43-46.

(2) Usually as GLEW at positions 44-47.

(3) Usually as KERE or KQRE at positions 43-46, e.g. as KEREL, KEREF, KQREL, KQREF, KEREG, KQREW or KQREG at positions 43-47. Alternatively, also sequences such as TERE (for example TEREL), TQRE (for example TQREL), KECE (for example KECEL or KECER), KQCE (for example KQCEL), RERE (for example REREG), RQRE (for example RQREL, RQREF or RQREW), QERE (for example QEREG), QQRE, (for example QQREW,

QQREL or QQREF), KGRE (for example KGREG), KDRE (for example KDREV) are possible. Some other possible, but less preferred sequences include for example DECKL and NVCEL.

(4) With both GLEW at positions 44-47 and KERE or KQRE at positions 43-46.

(5) Often as KP or EP at positions 83-84 of naturally occurring V_{HH} domains.

(6) In particular, but not exclusively, in combination with GLEW at positions 44-47.

(7) With the proviso that when positions 44-47 are GLEW, position 108 is always Q in (non-humanized) V_{HH} sequences that also contain a W at 103.

(8) The GLEW group also contains GLEW-like sequences at positions 44-47, such as for example GVEW, EPEW, GLER, DQEW, DLEW, GIEW, ELEW, GPEW, EWLP, GPER, GLER and ELEW.

Table B-3: Some preferred but non-limiting combinations of Hallmark Residues in naturally occurring Nanobodies®.

For humanization of these combinations, reference is made to the specification.

	11	37	44	45	47	83	84	103	104	108
DP-47 (human)	*M*	*V*	*G*	*L*	*W*	*R*	*A*	*W*	*G*	*L*
"KERE" group	L	F	E	R	L	K	P	W	G	Q
	L	F	E	R	F	E	P	W	G	Q
	L	F	E	R	F	K	P	W	G	Q
	L	Y	Q	R	L	K	P	W	G	Q
	L	F	L	R	V	K	P	Q	G	Q
	L	F	Q	R	L	K	P	W	G	Q
	L	F	E	R	F	K	P	W	G	Q
"GLEW" group	L	V	G	L	W	K	S	W	G	Q
	M	V	G	L	W	K	P	R	G	Q

In the Nanobodies®, each amino acid residue at any other position than the Hallmark Residues can be any amino acid residue that naturally occurs at the corresponding position (according to the Kabat numbering) of a naturally occurring V_{HH} domain.

Such amino acid residues will be clear to the skilled person. Tables B-4 to B-7 mention some non-limiting residues that can be present at each position (according to the Kabat numbering) of the FR1, FR2, FR3 and FR4 of naturally occurring V_{HH} domains. For each position, the amino acid residue that most frequently occurs at each position of a naturally occurring V_{HH} domain (and which is the most preferred amino acid residue for said position in a Nanobody®) is indicated in bold; and other preferred amino acid residues for each position have been underlined (note: the number of amino acid residues that are found at positions 26-30 of naturally occurring V_{HH} domains supports the hypothesis underlying the numbering by Chothia (supra) that the residues at these positions already form part of CDR1).

In Tables B-4 – B-7, some of the non-limiting residues that can be present at each position of a human V_H3 domain have also been mentioned. Again, for each position, the amino acid residue that most frequently occurs at each position of a naturally occurring human V_H3 domain is indicated in bold; and other preferred amino acid residues have been underlined.

For reference only, Tables B-4-B-7 also contain data on the V_{HH} entropy ("*V_{HH} Ent.*") and V_{HH} variability ("*V_{HH} Var.*") at each amino acid position for a representative sample of 7732 V_{HH} sequences (including a.o. data kindly provided by David Lutje Hulsing and Prof. Theo Verrips of Utrecht University). The values for the V_{HH} entropy and the V_{HH} variability provide a measure for the variability and degree of conservation of amino acid residues between the 7732 V_{HH} sequences analyzed: low values (i.e. <1, such as < 0.5) indicate that an amino acid residue is highly conserved between the V_{HH} sequences (i.e. little variability). For example, the G at position 9 and the W at position 36 have values for the V_{HH} entropy of 0.01 and 0 respectively, indicating that these residues are highly conserved and have little variability (and in case of position 36 is W in all 7732 sequences analysed), whereas for residues that form part of the CDR's generally values of 1.5 or more are found (data not shown). Note that the data represented below support the hypothesis that the amino acid residues at positions 27-30 and maybe even also at positions 93 and 94 already form part of the CDR's (although the invention is not limited to any specific hypothesis or explanation, and as mentioned above, herein the numbering according to Kabat is used). For a general explanation of sequence entropy, sequence variability and the methodology for determining the same, see Oliveira et al., PROTEINS: Structure, Function and Genetics, 52: 544-552 (2003).

Table B-4: Non-limiting examples of amino acid residues in FR1 (for the footnotes, see the footnotes to Table B-2)

Pos.	Amino acid residue(s):		V_{HH} Ent.	V_{HH} Var.
	Human V_H3	*Camelid V_{HH}'s*		
1	**E**, Q	**E**, Q, K, D, A, G, R	0,47	5
2	**V**	**V**, M, A, E, L	0,04	1
3	**Q**	**Q**, K, P, H, F, R	0,04	1
4	**L**	**L**, M, Q, P, R, F, V	0,02	1
5	**V**, L	**V**, Q, M, E, A, L, P, K, R	0,35	3
6	**E**	**E**, A, Q, D, K, H	0,21	5
7	**S**, T	**S**, F, L, W, T	0,05	2
8	**G**, R	**G**, R, E, V	0,04	1
9	**G**	**G**, R, V, A	0,01	1
10	**G**, V	**G**, D, R, S, K, E, A, Q, N, T, V	0,22	4
11	**Hallmark residue:** L, S, V, M, W, F, T, Q, E, A, R, G, K, Y, N, P, I; preferably L		0,35	4
12	**V**, I	**V**, A, L, M, E, G, T	0,11	2
13	**Q**, K, R	**Q**, L, R, H, P, E, K, T, S, V, D, G, A, N, M	0,46	3
14	**P**	**A**, P, T, V, S, D, F, N, I, E, L, R, G, Y, Q, H	0,92	5
15	**G**	**G**, E	0	1
16	**G**, R	**G**, D, E, A, S, N, V, R, K, T, P, C, L	0,47	4
17	**S**	**S**, F, P, Y, T, A, C, R, N	0,14	2
18	**L**	**L**, V, R, M, P, Q, S, A, T, K, H	0,06	1
19	**R**, K	**R**, T, K, S, N, G, A, I, L, Q, F, E, V, M	0,36	4
20	**L**	**L**, F, V, I, P, H, S	0,18	3
21	**S**	**S**, A, T, P, F, V, H, D, R, L, I, G	0,13	3
22	**C**	**C**, W	0	1
23	**A**, T	**A**, V, T, E, S, L, G, I, K, Q, R, D, F, N, P, M	0,88	5
24	**A**	**A**, D, V, T, H, Y, P, G, S, F, L, I, N, Q, E, R	0,78	9

Table B-4: Non-limiting examples of amino acid residues in FR1 (continued)

Pos.	Amino acid residue(s):		V_{HH} Ent.	V_{HH} Var.
	Human V_H3	*Camelid V_{HH}'s*		
25	**S**	**S**, P, T, A, F, L, N, Y, R, H, D, V, I, W, G, K, Q, C	0,2	2
26	**G**	**G**, E, R, V, T, A, S, K, D, L, I, Q, N, F, Y, M, W, P, H	0,45	6
27	**F**	**R**, F, S, P, L, G, I, N, T, D, H, V, E, A, Y, K, M, Q, W, C	1,89	12
28	**T**	**T**, I, S, A, P, F, D, N, V, R, M, L, G, Y, K, E, H, W, Q	1,29	12
29	**F**, V	**F**, L, S, V, I, A, W, Y, G, D, R, T, P, N, E, M, H, Q, K, C	1,23	11
30	**S**, D, G	**S**, D, N, G, R, T, A, E, I, Y, K, V, H, L, F, W, M, P, C, Q	1,55	12

Table B-5: Non-limiting examples of amino acid residues in FR2 (for the footnotes, see the footnotes to Table B-2)

Pos.	Amino acid residue(s):		V_{HH} Ent.	V_{HH} Var.
	Human V_H3	*Camelid V_{HH}'s*		
36	**W**	**W**	0	1
37	**Hallmark residue:** $F^{(1)}$, Y, V, L, A, H, S, I, W, C, N, G, D, T, P , preferably $F^{(1)}$ or Y		1,1	7
38	**R**	**R**, H, C, P, Y, L, V	0,01	1
39	**Q**	**Q**, E, R, H, L, A, S, K, P, V, T, D	0,22	3
40	**A**	**A**, V, T, P, G, S, D, I, L, R, N, F, Y, C, E, H	0,55	6
41	**P**, S, T	**P**, S, A, L, T, Q, R, V, D, G, I, H	0,18	3
42	**G**	**G**, E, A, R, D, V, W, T, Q, K, L, N, H, M	0,1	2
43	**K**	**K**, N, Q, E, R, T, L, S, M, D, G, A, V, H, I, F, P	0,45	7
44	**Hallmark residue:** $E^{(3)}$, $Q^{(3)}$, $G^{(2)}$, D, A, K, R, L, P, S, V, H, T, N, W, M, I; preferably $G^{(2)}$, $E^{(3)}$ or $Q^{(3)}$; most preferably $G^{(2)}$ or $Q^{(3)}$		1,11	4
45	**Hallmark residue**: $L^{(2)}$, $R^{(3)}$, P, H, F, G, Q, S, E, T, Y, C, I, D, V; preferably $L^{(2)}$ or $R^{(3)}$		0,56	3
46	E, V	E, D, A, Q, V, M, K, T, G, R, S, N, I, L, F	0,42	4
47	**Hallmark residue:** $F^{(1)}$, $L^{(1)}$ or $W^{(2)}$ G, I, S, A, V, M, R, Y, E, P, T, C, H, K, Q, N, D; preferably $W^{(2)}$, $L^{(1)}$ or $F^{(1)}$		1,64	11
48	**V**	**V**, I, L, A, T, Q, F, M, G, E, R	0,35	5
49	**S**, A, G	**A**, S, G, T, V, L, C, I, F, P, E, Y, M, D, R	0,89	5

Table B-6: Non-limiting examples of amino acid residues in FR3 (for the footnotes, see the footnotes to Table B-2)

Pos.	Amino acid residue(s):		V_{HH} Ent.	V_{HH} Var.
	Human V_H3	*Camelid V_{HH}'s*		
66	**R**	**R**	0	1
67	**F**	**F**, S, L, V, I, C, A, Y, M, G	0,1	1
68	**T**	**T**, A, S, I, F, V, P, N, G, R, K, M, D, L, W, Q	0,34	4
69	**I**	**I**, V, M, T, L, A, F, P, S, G, N	0,5	5
70	**S**	**S**, T, A, F, P, V, Y, L, D, G, N, H, W, E, C	0,22	4
71	**R**	**R**, S, K, G, T, I, W, A, N, V, E, L, M, F, D, Q, C	0,61	7
72	**D**, E	**D**, N, E, G, V, A, H, L, S, T, I, Q, F, P, Y, R	0,34	4
73	**N**, D, G	**N**, D, S, K, I, Y, G, T, H, R, A, V, F, L, E, M, P, C	0,65	9
74	**A**, S	**A**, T, V, S, F, G, D, P, N, I, R, L, Y, H, E, Q, K, W, M	0,8	8
75	**K**	**K**, N, E, R, Q, A, G, T, M, S, L, D, V, W, Y, I	0,71	6
76	**N**, S	**N**, K, S, R, D, T, H, G, E, A, Y, I, M, Q, L, W, P, F, V	0,66	7
77	S, T, I	**T**, A, M, S, R, I, V, L, P, E, N, K, G, W, Q	0,72	7
78	**L**, A	**V**, L, A, M, I, G, T, F, W, Q, S, E, N, H	1,11	6
79	**Y**, H	**Y**, F, D, S, H, N, T, A, L, W, V, C, G, E, I, P, R	0,68	8
80	**L**	**L**, M, V, P, F	0,05	2
81	**Q**	**Q**, E, R, H, L, D, T, G, K, P, A, I, S, N, Y, V, M	0,38	4
82	**M**	**M**, I, L, V, A, T, S, K	0,12	3
82a	**N**, G	**N**, S, D, T, E, H, K, I, A, G, R, Y, L, V, F, Q	0,77	5
82b	**S**	**S**, N, T, G, H, D, R, A, K, I, M, V, F, E, P, Y, C, L	0,72	8
82c	**L**	**L**, V, M, P, A, T, G	0,08	2
83	**Hallmark residue:** R, K[5], T, E[5], Q, N, S, I, V, G, M, L, A, D, Y, H; preferably K or R; most preferably K		0,66	6
84	**Hallmark residue:** P[5], S, H, L, A, V, I, T, F, D, R, Y, N, Q, G, E; preferably P		0,85	7
85	**E**, G	**E**, D, G, A, Q, V, S, N, K, T, R, L	0,27	3
86	**D**	**D**, E, G, N	0,02	1
87	**T**, M	**T**, S, A, M, R, P, K, E	0,15	3

Table B-6: Non-limiting examples of amino acid residues in FR3 (continued)

Pos.	Amino acid residue(s):		V_{HH} Ent.	V_{HH} Var.
	Human V_H3	*Camelid V_{HH}'s*		
88	**A**	**A**, G, S, D, N, T, P, V	0,23	2
89	**V**, L	**V**, I, L, E, A, R, T, D, F, M, N, S, K, G, Q, H	0,71	7
90	**Y**	**Y**, H, F, N	0	1
91	**Y**, H	**Y**, F, R, S, H, T, I, V, L, N, D, C, Q, W, A, E, M	0,6	7
92	**C**	**C**, R, P	0	1
93	**A**, K, T	**A**, N, T, K, G, V, R, Y, S, H, W, L, F, Q, M, I, E, C, D	1,33	10
94	**K**, R, T	**A**, K, V, T, R, L, G, S, D, Q, I, M, F, Y, N, E, H, P, C, W	1,55	12

Table B-7: Non-limiting examples of amino acid residues in FR4 (for the footnotes, see the footnotes to Table B-2)

Pos.	Amino acid residue(s):		V_{HH} Ent.	V_{HH} Var.
	Human V_H3	*Camelid V_{HH}'s*		
103	**Hallmark residue:** W[4], R[6], G, S, K, A, M, Y, L, F, T, N, V, Q, P[6], E, C; preferably W		0,54	6
104	**Hallmark residue:** G, A, S, T, D, P, N, E, C, L; preferably G		0,13	3
105	**Q**, R	**Q**, K, H, R, P, E, L, T, N, S, V, A, M, G	0,52	5
106	**G**	**G**, R, E	0	1
107	**T**	**T**, Q, I, A, S, N, R, V, D	0,24	3
108	**Hallmark residue:** Q, L[7], R, P, E, K, S, T, M, A, H; preferably Q or L[7]		0,3	4
109	**V**	**V**, I, L	0	1
110	**T**	**T**, S, N, A, I, F	0,01	1
111	**V**	**V**, I, A	0,01	1
112	**S**	**S**, T, P, F, A	0,01	1
113	**S**	**S**, T, A, L, P, F, E, V	0,04	1

Thus, in another preferred, but not limiting aspect, a Nanobody® of the invention can be defined as an amino acid sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2;

and in which:

ii) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

The above Nanobodies® may for example be V_{HH} sequences or may be humanized Nanobodies®. When the above Nanobody® sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies® are partially humanized Nanobodies®, they may optionally be further suitably humanized, again as described herein.

In particular, a Nanobody® of the invention can be an amino acid sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) (preferably) one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2 (it being understood that V_{HH} sequences will contain one or more Hallmark residues; and that partially humanized Nanobodies® will usually, and preferably, [still] contain one or more Hallmark residues [although it is also within the scope of the invention to provide - where suitable in accordance with the invention - partially humanized Nanobodies® in which all Hallmark residues, but not one or more of the other amino acid residues, have been humanized]; and that in fully humanized Nanobodies®, where suitable in accordance with the invention, all amino acid residues at the positions of the Hallmark residues will be amino

acid residues that occur in a human V_H3 sequence. As will be clear to the skilled person based on the disclosure herein that such V_{HH} sequences, such partially humanized Nanobodies® with at least one Hallmark residue, such partially humanized Nanobodies® without Hallmark residues and such fully humanized Nanobodies® all form aspects of this invention);

and in which:

ii) said amino acid sequence has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1 to 22, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences (indicated with X in the sequences of SEQ ID NO's: 1 to 22) are disregarded;

and in which:

iii) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

The above Nanobodies® may for example be V_{HH} sequences or may be humanized Nanobodies®. When the above Nanobody® sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies® are partially humanized Nanobodies®, they may optionally be further suitably humanized, again as described herein.

Table B-8: Representative amino acid sequences for Nanobodies® of the KERE, GLEW and P,R,S 103 group.

The CDR's are indicated with XXXX

KERE sequence no. 1	SEQ ID NO:1	EVQLVESGGGLVQPGGSLRLSCAASGIPFSXXXXXWFRQAPGKQRDSVAXXXXXRFTISR DNAKNTVYLQMNSLKPEDTAVYRCYFXXXXXWGQGTQVTVSS
KERE sequence no. 2	SEQ ID NO:2	QVKLEESGGGLVQAGGSLRLSCVGSGRTFSXXXXXWFRLAPGKEREFVAXXXXXRFTISR DTASNRGYLHMNNLTPEDTAVYYCAAXXXXXWGQGTQVTVSS
KERE sequence no. 3	SEQ ID NO:3	AVQLVDSGGGLVQAGDSLKLSCALTGGAFTXXXXXWFRQTPGREREFVAXXXXXRFTIS RDNAKNMVYLRMNSLIPEDAAVYSCAAXXXXXWGQGTLVTVSS
KERE sequence no. 4	SEQ ID NO:4	QVQLVESGGGLVEAGGSLRLSCTASESPFRXXXXXWFRQTSGQEREFVAXXXXXRFTISR DDAKNTVWLHGSTLKPEDTAVYYCAAXXXXXWGQGTQVTVSS
KERE sequence no. 5	SEQ ID NO:5	AVQLVESGGGLVQGGGSLRLACAASERIFDXXXXXWYRQGPGNERELVAXXXXXRFTIS MDYTKQTVYLHMNSLRPEDTGLYYCKIXXXXXWGQGTQVTVSS
KERE sequence no. 6	SEQ ID NO:6	DVKFVESGGGLVQAGGSLRLSCVASGFNFDXXXXXWFRQAPGKEREEVAXXXXXRFTIS SEKDKNSVYLQMNSLKPEDTALYICAGXXXXXWGRGTQVTVSS
KERE sequence no. 7	SEQ ID NO:7	QVRLAESGGGLVQSGGSLRLSCVASGSTYTXXXXXWYRQYPGKQRALVAXXXXXRFTIA RDSTKDTFCLQMNNLKPEDTAVYYCYAXXXXXWGQGTQVTVSS
KERE sequence no. 8	SEQ ID NO:8	EVQLVESGGGLVQAGGSLRLSCAASGFTSDXXXXXWFRQAPGKPREGVSXXXXXRFTIST DNAKNTVHLLMNRVNAEDTALYYCAVXXXXXWGRGTRVTVSS
KERE sequence no. 9	SEQ ID NO:9	QVQLVESGGGLVQPGGSLRLSCQASGDISTXXXXXWYRQVPGKLREFVAXXXXXRFTISG DNAKRAIYLQMNNLKPDDTAVYYCNRXXXXXWGQGTQVTVSP
KERE sequence no. 10	SEQ ID NO:10	QVPVVESGGGLVQAGDSLRLFCAVPSFTSTXXXXXWFRQAPGKEREFVAXXXXXRFTISR NATKNTLTLRMDSLKPEDTAVYYCAAXXXXXWGQGTQVTVSS

KERE sequence no. 11	SEQ ID NO:11	EVQLVESGGGLVQAGDSLRLFCTVSGGTASXXXXWFRQAPGEKREFVAXXXXXRFTIA RENAGNMVYLQMNNLKPDDTALYTCAAXXXXXWGRGTQVTVSS

Table B-8 (continued):

KERE sequence no. 12	SEQ ID NO:12	AVQLVESGGDSVQPGDSQTLSCAASGRTNSXXXXXWFRQAPGKERVFLAXXXXXRFTIS RDSAKNMMYLQMNNLKPQDTAVYYCAAXXXXXWGQGTQVTVSS
KERE sequence no. 13	SEQ ID NO:13	AVQLVESGGGLVQAGGSLRLSCVVSGLTSSXXXXXWFRQTPWQERDFVAXXXXXRFTIS RDNYKDTVLLEMNFLKPEDTAIYYCAAXXXXXWGQGTQVTVSS
KERE sequence no. 14	SEQ ID NO:14	AVQLVESGGGLVQAGASLRLSCATSTRTLDXXXXXWFRQAPGRDREFVAXXXXXRFTVS RDSAENTVALQMNSLKPEDTAVYYCAAXXXXXWGQGTRVTVSS
KERE sequence no. 15	SEQ ID NO:15	QVQLVESGGGLVQPGGSLRLSCTVSRLTAHXXXXXWFRQAPGKEREAVSXXXXXRFTISR DYAGNTAFLQMDSLKPEDTGVYYCATXXXXXWGQGTQVTVSS
KERE sequence no. 16	SEQ ID NO:16	EVQLVESGGELVQAGGSLKLSCTASGRNFVXXXXXWFRRAPGKEREFVAXXXXXRFTVS RDNGKNTAYLRMNSLKPEDTADYYCAVXXXXXLGSGTQVTVSS
GLEW sequence no. 1	SEQ ID NO:17	AVQLVESGGGLVQPGGSLRLSCAASGFTFSXXXXXWVRQAPGKVLEWVSXXXXXRFTIS RDNAKNTLYLQMNSLKPEDTAVYYCVKXXXXXGSQGTQVTVSS
GLEW sequence no. 2	SEQ ID NO:18	EVQLVESGGGLVQPGGSLRLSCVCVSSGCTXXXXXWVRQAPGKAEEWVSXXXXXRFKIS RDNAKKTLYLQMNSLGPEDTAMYYCQRXXXXXRGQGTQVTVSS
GLEW sequence no. 3	SEQ ID NO:19	EVQLVESGGGLALPGGSLTLSCVFSGSTFSXXXXXWVRHTPGKAEEWVSXXXXXRFTISR DNAKNTLYLEMNSLSPEDTAMYYCGRXXXXXRSKGIQVTVSS
P,R,S 103 sequence no. 1	SEQ ID NO:20	AVQLVESGGGLVQAGGSLRLSCAASGRTFSXXXXXWFRQAPGKEREFVAXXXXXRFTISR DNAKNTVYLQMNSLKPEDTAVYYCAAXXXXXRGQGTQVTVSS
P,R,S 103 sequence no. 2	SEQ ID NO:21	DVQLVESGGDLVQPGGSLRLSCAASGFSFDXXXXXWLRQTPGKGLEWVGXXXXXRFTIS RDNAKNMLYLHLNNLKSEDTAVYYCRRXXXXXLGQGTQVTVSS

P,R,S 103 sequence no. 3	SEQ ID NO:22	EVQLVESGGGLVQPGGSLRLSCVCVSSGCTXXXXXWVRQAPGKAEEWVSXXXXXRFKIS RDNAKKTLYLQMNSLGPEDTAMYYCQRXXXXXRGQGTQVTVSS

In particular, a Nanobody® of the invention of the KERE group can be an amino acid sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which:

i) the amino acid residue at position 45 according to the Kabat numbering is a charged amino acid (as defined herein) or a cysteine residue, and position 44 is preferably an E;

and in which:

ii) FR1 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-9: Representative FW1 sequences for Nanobodies® of the KERE-group.

KERE FW1 sequence no. 1	SEQ ID NO:23	QVQRVESGGGLVQAGGSLRLSCAASGRTSS
KERE FW1 sequence no. 2	SEQ ID NO:24	QVQLVESGGGLVQTGDSLSLSCSASGRTFS
KERE FW1 sequence no. 3	SEQ ID NO:25	QVKLEESGGGLVQAGDSLRLSCAATGRAFG
KERE FW1 sequence no. 4	SEQ ID NO:26	AVQLVESGGGLVQPGESLGLSCVASGRDFV
KERE FW1 sequence no. 5	SEQ ID NO:27	EVQLVESGGGLVQAGGSLRLSCEVLGRTAG
KERE FW1 sequence no. 6	SEQ ID NO:28	QVQLVESGGGWVQPGGSLRLSCAASETILS
KERE FW1 sequence no. 7	SEQ ID NO:29	QVQLVESGGGTVQPGGSLNLSCVASGNTFN
KERE FW1 sequence no. 8	SEQ ID NO:30	EVQLVESGGGLAQPGGSLQLSCSAPGFTLD
KERE FW1 sequence no. 9	SEQ ID NO:31	AQELEESGGGLVQAGGSLRLSCAASGRTFN

and in which:

iii) FR2 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-10: Representative FW2 sequences for Nanobodies® of the KERE-group.

KERE FW2 sequence no. 1	SEQ ID NO:41	WFRQAPGKEREFVA
KERE FW2 sequence no. 2	SEQ ID NO:42	WFRQTPGREREFVA
KERE FW2 sequence no. 3	SEQ ID NO:43	WYRQAPGKQREMVA
KERE FW2 sequence no. 4	SEQ ID NO:44	WYRQGPGKQRELVA
KERE FW2 sequence no. 5	SEQ ID NO:45	WIRQAPGKEREGVS
KERE FW2 sequence no. 6	SEQ ID NO:46	WFREAPGKEREGIS
KERE FW2 sequence no. 7	SEQ ID NO:47	WYRQAPGKERDLVA
KERE FW2 sequence no. 8	SEQ ID NO:48	WFRQAPGKQREEVS
KERE FW2 sequence no. 9	SEQ ID NO:49	WFRQPPGKVREFVG

and in which:

iv) FR3 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-11: Representative FW3 sequences for Nanobodies® of the KERE-group.

KERE FW3 sequence no. 1	SEQ ID NO:50	RFTISRDNAKNTVYLQMNSLKPEDTAVYRCYF
KERE FW3 sequence no. 2	SEQ ID NO:51	RFAISRDNNKNTGYLQMNSLEPEDTAVYYCAA
KERE FW3 sequence no. 3	SEQ ID NO:52	RFTVARNNAKNTVNLEMNSLKPEDTAVYYCAA
KERE FW3 sequence no. 4	SEQ ID NO:53	RFTISRDIAKNTVDLLMNNLEPEDTAVYYCAA
KERE FW3 sequence no. 5	SEQ ID NO:54	RLTISRDNAVDTMYLQMNSLKPEDTAVYYCAA
KERE FW3 sequence no. 6	SEQ ID NO:55	RFTISRDNAKNTVYLQMDNVKPEDTAIYYCAA
KERE FW3 sequence no. 7	SEQ ID NO:56	RFTISKDSGKNTVYLQMTSLKPEDTAVYYCAT
KERE FW3 sequence no. 8	SEQ ID NO:57	RFTISRDSAKNMMYLQMNNLKPQDTAVYYCAA
KERE FW3 sequence no. 9	SEQ ID NO:58	RFTISRENDKSTVYLQLNSLKPEDTAVYYCAA
KERE FW3 sequence no. 10	SEQ ID NO:59	RFTISRDYAGNTAYLQMNSLKPEDTGVYYCAT

and in which:

v) FR4 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-12: Representative FW4 sequences for Nanobodies® of the KERE-group.

KERE FW4 sequence no. 1	SEQ ID NO:60	WGQGTQVTVSS
KERE FW4 sequence no. 2	SEQ ID NO:61	WGKGTLVTVSS
KERE FW4 sequence no. 3	SEQ ID NO:62	RGQGTRVTVSS
KERE FW4 sequence no. 4	SEQ ID NO:63	WGLGTQVTISS

and in which:

vi) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

In the above Nanobodies®, one or more of the further Halimark residues are preferably as described herein (for example, when they are V_{HH} sequences or partially humanized Nanobodies®).

Also, the above Nanobodies® may for example be V_{HH} sequences or may be humanized Nanobodies®. When the above Nanobody® sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies® are partially humanized Nanobodies®, they may optionally be further suitably humanized, again as described herein.

With regard to framework 1, it will be clear to the skilled person that, when an amino acid sequence as outlined above is generated by expression of a nucleotide sequence, the first four amino acid sequences (i.e. amino acid residues 1-4 according to the Kabat numbering) may often be determined by the primer(s) that have been used to generate said nucleic acid. Thus, for determining the degree of amino acid identity, the first four amino acid residues are preferably disregarded.

Also, with regard to framework 1, and although amino acid positions 27 to 30 are according to the Kabat numbering considered to be part of the framework regions (and not the CDR's), it has been found by analysis of a database of more than 1000 V_{HH} sequences that the positions 27 to 30 have a variability (expressed in terms of V_{HH} entropy and V_{HH} variability – see Tables B-4 to B-7) that is much greater than the variability on positions 1 to 26. Because of this, for determining the degree of amino acid identity, the amino acid residues at positions 27 to 30 are preferably also disregarded.

In view of this, a Nanobody® of the KERE class may be an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which:

i) the amino acid residue at position 45 according to the Kabat numbering is a charged amino acid (as defined herein) or a cysteine residue, and position 44 is preferably an E;

and in which:

ii) FR1 is an amino acid sequence that, on positions 5 to 26 of the Kabat numbering, has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-13: Representative FW1 sequences (amino acid residues 5 to 26) for Nanobodies® of the KERE-group.

KERE FW1 sequence no. 10	SEQ ID NO:32	VESGGGLVQPGGSLRLSCAASG
KERE FW1 sequence no. 11	SEQ ID NO:33	VDSGGGLVQAGDSLKLSCALTG
KERE FW1 sequence no. 12	SEQ ID NO:34	VDSGGGLVQAGDSLRLSCAASG
KERE FW1 sequence no. 13	SEQ ID NO:35	VDSGGGLVEAGGSLRLSCQVSE
KERE FW1 sequence no. 14	SEQ ID NO:36	QDSGGGSVQAGGSLKLSCAASG
KERE FW1 sequence no. 15	SEQ ID NO:37	VQSGGRLVQAGDSLRLSCAASE
KERE FW1 sequence no. 16	SEQ ID NO:38	VESGGTLVQSGDSLKLSCASST
KERE FW1 sequence no. 17	SEQ ID NO:39	MESGGDSVQSGGSLTLSCVASG
KERE FW1 sequence no. 18	SEQ ID NO:40	QASGGGLVQAGGSLRLSCSASV

and in which:

iii) FR2, FR3 and FR4 are as mentioned herein for FR2, FR3 and FR4 of Nanobodies® of the KERE-class;

and in which:

iv) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

The above Nanobodies® may for example be V_{HH} sequences or may be humanized Nanobodies®. When the above Nanobody® sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies® are partially humanized Nanobodies®, they may optionally be further suitably humanized, again as described herein.

A Nanobody® of the GLEW class may be an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which

i) preferably, when the Nanobody® of the GLEW-class is a non-humanized Nanobody®, the amino acid residue in position 108 is Q;

ii) FR1 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-14: Representative FW1 sequences for Nanobodies® of the GLEW-group.

GLEW FW1 sequence no. 1	SEQ ID NO:64	QVQLVESGGGLVQPGGSLRLSCAASGFTFS
GLEW FW1 sequence no. 2	SEQ ID NO:65	EVHLVESGGGLVRPGGSLRLSCAAFGFIFK
GLEW FW1 sequence no. 3	SEQ ID NO:66	QVKLEESGGGLAQPGGSLRLSCVASGFTFS
GLEW FW1 sequence no. 4	SEQ ID NO:67	EVQLVESGGGLVQPGGSLRLSCVCVSSGCT
GLEW FW1 sequence no. 5	SEQ ID NO:68	EVQLVESGGGLALPGGSLTLSCVFSGSTFS

and in which:

iii) FR2 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-15: Representative FW2 sequences for Nanobodies® of the GLEW-group.

GLEW FW2 sequence no. 1	SEQ ID NO:72	WVRQAPGKVLEWVS
GLEW FW2 sequence no. 2	SEQ ID NO:73	WVRRPPGKGLEWVS
GLEW FW2 sequence no. 3	SEQ ID NO:74	WVRQAPGMGLEWVS
GLEW FW2 sequence no. 4	SEQ ID NO:75	WVRQAPGKEPEWVS
GLEW FW2 sequence no. 5	SEQ ID NO:76	WVRQAPGKDQEWVS
GLEW FW2 sequence no. 6	SEQ ID NO:77	WVRQAPGKAEEWVS
GLEW FW2 sequence no. 7	SEQ ID NO:78	WVRQAPGKGLEWVA
GLEW FW2 sequence no. 8	SEQ ID NO:79	WVRQAPGRATEWVS

and in which:

iv) FR3 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-16: Representative FW3 sequences for Nanobodies® of the GLEW-group.

GLEW FW3 sequence no. 1	SEQ ID NO:80	RFTISRDNAKNTLYLQMNSLKPEDTAVYYCVK
GLEW FW3 sequence no. 2	SEQ ID NO:81	RFTISRDNARNTLYLQMDSLIPEDTALYYCAR
GLEW FW3 sequence no. 3	SEQ ID NO:82	RFTSSRDNAKSTLYLQMNDLKPEDTALYYCAR
GLEW FW3 sequence no. 4	SEQ ID NO:83	RFIISRDNAKNTLYLQMNSLGPEDTAMYYCQR
GLEW FW3 sequence no. 5	SEQ ID NO:84	RFTASRDNAKNTLYLQMNSLKSEDTARYYCAR
GLEW FW3 sequence no. 6	SEQ ID NO:85	RFTISRDNAKNTLYLQMDDLQSEDTAMYYCGR

and in which:

v) FR4 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-17: Representative FW4 sequences for Nanobodies® of the GLEW-group.

GLEW FW4 sequence no. 1	SEQ ID NO:86	GSQGTQVTVSS
GLEW FW4 sequence no. 2	SEQ ID NO:87	LRGGTQVTVSS
GLEW FW4 sequence no. 3	SEQ ID NO:88	RGQGTLVTVSS
GLEW FW4 sequence no. 4	SEQ ID NO:89	RSRGIQVTVSS
GLEW FW4 sequence no. 5	SEQ ID NO:90	WGKGTQVTVSS
GLEW FW4 sequence no. 6	SEQ ID NO:91	WGQGTQVTVSS

and in which:

vi) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

In the above Nanobodies®, one or more of the further Hallmark residues are preferably as described herein (for example, when they are V_{HH} sequences or partially humanized Nanobodies®).

With regard to framework 1, it will again be clear to the skilled person that, for determining the degree of amino acid identity, the amino acid residues on positions 1 to 4 and 27 to 30 are preferably disregarded.

In view of this, a Nanobody® of the GLEW class may be an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which:

i) preferably, when the Nanobody® of the GLEW-class is a non-humanized Nanobody®, the amino acid residue in position 108 is Q;

and in which:

ii) FR1 is an amino acid sequence that, on positions 5 to 26 of the Kabat numbering, has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-18: Representative FW1 sequences (amino acid residues 5 to 26) for Nanobodies® of the KERE-group.

GLEW FW1 sequence no. 6	SEQ ID NO:69	VESGGGLVQPGGSLRLSCAASG
GLEW FW1 sequence no. 7	SEQ ID NO:70	EESGGGLAQPGGSLRLSCVASG
GLEW FW1 sequence no. 8	SEQ ID NO:71	VESGGGLALPGGSLTLSCVFSG

and in which:

iii) FR2, FR3 and FR4 are as mentioned herein for FR2, FR3 and FR4 of Nanobodies® of the GLEW-class;

and in which:

iv) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

The above Nanobodies® may for example be V_{HH} sequences or may be humanized Nanobodies®. When the above Nanobody® sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies® are partially humanized Nanobodies®, they may optionally be further suitably humanized, again as described herein. In the above Nanobodies®, one or more of the further Hallmark residues are preferably as described herein (for example, when they are V_{HH} sequences or partially humanized Nanobodies®).

A Nanobody® of the P, R, S 103 class may be an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which

i) the amino acid residue at position 103 according to the Kabat numbering is different from W;

and in which:

ii) preferably the amino acid residue at position 103 according to the Kabat numbering is P, R or S, and more preferably R;

and in which:

iii) FR1 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-19: Representative FW1 sequences for Nanobodies® of the P,R,S 103-group.

P,R,S 103 FW1 sequence no. 1	SEQ ID NO:92	AVQLVESGGGLVQAGGSLRLSCAASGRTFS
P,R,S 103 FW1 sequence no. 2	SEQ ID NO:93	QVQLQESGGGMVQPGGSLRLSCAASGFDFG
P,R,S 103 FW1 sequence no. 3	SEQ ID NO:94	EVHLVESGGGLVRPGGSLRLSCAAFGFIFK
P,R,S 103 FW1 sequence no. 4	SEQ ID NO:95	QVQLAESGGGLVQPGGSLKLSCAASRTIVS
P,R,S 103 FW1 sequence no. 5	SEQ ID NO:96	QEHLVESGGGLVDIGGSLRLSCAASERIFS
P,R,S 103 FW1 sequence no. 6	SEQ ID NO:97	QVKLEESGGGLAQPGGSLRLSCVASGFTFS
P,R,S 103 FW1 sequence no. 7	SEQ ID NO:98	EVQLVESGGGLVQPGGSLRLSCVCVSSGCT
P,R,S 103 FW1 sequence no. 8	SEQ ID NO:99	EVQLVESGGGLALPGGSLTLSCVFSGSTFS

and in which

iv) FR2 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-20: Representative FW2 sequences for Nanobodies® of the P,R,S 103-group.

P,R,S 103 FW2 sequence no. 1	SEQ ID NO:102	WFRQAPGKEREFVA
P,R,S 103 FW2 sequence no. 2	SEQ ID NO:103	WVRQAPGKVLEWVS
P,R,S 103 FW2 sequence no. 3	SEQ ID NO:104	WVRRPPGKGLEWVS
P,R,S 103 FW2 sequence no. 4	SEQ ID NO:105	WIRQAPGKEREGVS
P,R,S 103 FW2 sequence no. 5	SEQ ID NO:106	WVRQYPGKEPEWVS
P,R,S 103 FW2 sequence no. 6	SEQ ID NO:107	WFRQPPGKEHEFVA
P,R,S 103 FW2 sequence no. 7	SEQ ID NO:108	WYRQAPGKRTELVA
P,R,S 103 FW2 sequence no. 8	SEQ ID NO:109	WLRQAPGQGLEWVS
P,R,S 103 FW2 sequence no. 9	SEQ ID NO:110	WLRQTPGKGLEWVG
P,R,S 103 FW2 sequence no. 10	SEQ ID NO:111	WVRQAPGKAEEFVS

and in which:

v) FR3 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-21: Representative FW3 sequences for Nanobodies® of the P,R,S 103-group.

P,R,S 103 FW3 sequence no. 1	SEQ ID NO:112	RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA
P,R,S 103 FW3 sequence no. 2	SEQ ID NO:113	RFTISRDNARNTLYLQMDSLIPEDTALYYCAR
P,R,S 103 FW3 sequence no. 3	SEQ ID NO:114	RFTISRDNAKNEMYLQMNNLKTEDTGVYWCGA
P,R,S 103 FW3 sequence no. 4	SEQ ID NO:115	RFTISSDSNRNMIYLQMNNLKPEDTAVYYCAA
P,R,S 103 FW3 sequence no. 5	SEQ ID NO:116	RFTISRDNAKNMLYLHLNNLKSEDTAVYYCRR
P,R,S 103 FW3 sequence no. 6	SEQ ID NO:117	RFTISRDNAKKTVYLRLNSLNPEDTAVYSCNL
P,R,S 103 FW3 sequence no. 7	SEQ ID NO:118	RFKISRDNAKKTLYLQMNSLGPEDTAMYYCQR
P,R,S 103 FW3 sequence no. 8	SEQ ID NO:119	RFTVSRDNGKNTAYLRMNSLKPEDTADYYCAV

and in which:

vi) FR4 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-22: Representative FW4 sequences for Nanobodies® of the P,R,S 103-group.

P,R,S 103 FW4 sequence no. 1	SEQ ID NO:120	RGQGTQVTVSS
P,R,S 103 FW4 sequence no. 2	SEQ ID NO:121	LRGGTQVTVSS
P,R,S 103 FW4 sequence no. 3	SEQ ID NO:122	GNKGTLVTVSS
P,R,S 103 FW4 sequence no. 4	SEQ ID NO:123	SSPGTQVTVSS
P,R,S 103 FW4 sequence no. 5	SEQ ID NO:124	SSQGTLVTVSS
P,R,S 103 FW4 sequence no. 6	SEQ ID NO:125	RSRGIQVTVSS

and in which:

vii) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

In the above Nanobodies®, one or more of the further Hallmark residues are preferably as described herein (for example, when they are V_{HH} sequences or partially humanized Nanobodies®).

With regard to framework 1, it will again be clear to the skilled person that, for determining the degree of amino acid identity, the amino acid residues on positions 1 to 4 and 27 to 30 are preferably disregarded.

In view of this, a Nanobody® of the P,R,S 103 class may be an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which:

i) the amino acid residue at position 103 according to the Kabat numbering is different from W;

and in which:

ii) preferably the amino acid residue at position 103 according to the Kabat numbering is P, R or S, and more preferably R;

and in which:

iii) FR1 is an amino acid sequence that, on positions 5 to 26 of the Kabat numbering, has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table B-23: Representative FW1 sequences (amino acid residues 5 to 26) for Nanobodies® of the P,R,S 103-group.

P,R,S 103 FW1 sequence no. 9	SEQ ID NO:100	VESGGGLVQAGGSLRLSCAASG
P,R,S 103 FW1 sequence no. 10	SEQ ID NO:101	AESGGGLVQPGGSLKLSCAASR

and in which:

iv) FR2, FR3 and FR4 are as mentioned herein for FR2, FR3 and FR4 of Nanobodies® of the P,R,S 103 class;

and in which:

v) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

The above Nanobodies® may for example be V_{HH} sequences or may be humanized Nanobodies®. When the above Nanobody® sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies® are partially humanized Nanobodies®, they may optionally be further suitably humanized, again as described herein.

In the above Nanobodies®, one or more of the further Hallmark residues are preferably as described herein (for example, when they are V_{HH} sequences or partially humanized Nanobodies®).

In another preferred, but non-limiting aspect, the invention relates to a Nanobody® as described above, in which the CDR sequences have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1). This degree of amino acid identity can for example be determined by determining the degree of amino acid identity (in a manner described herein) between said Nanobody® and one or more of the sequences of SEQ ID NO's: 1589-1797 (see Table A-1), in which the amino acid residues that form the framework regions are disregarded. Such Nanobodies® can be as further described herein.

As already mentioned herein, another preferred but non-limiting aspect of the invention relates to a Nanobody® with an amino acid sequence that is chosen from the group consisting of SEQ ID NO's: 1589-1797 (see Table A-1) or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 (see Table A-1).

Also, in the above Nanobodies®:

i) any amino acid substitution (when it is not a humanizing substitution as defined herein) is preferably, and compared to the corresponding amino acid sequence of SEQ ID NO's: 1589-1797 (see Table A-1), a conservative amino acid substitution, (as defined herein);

and/or:

ii) its amino acid sequence preferably contains either only amino acid substitutions, or otherwise preferably no more than 5, preferably no more than 3, and more preferably only 1 or 2 amino acid deletions or insertions, compared to the corresponding amino acid sequence of SEQ ID NO's: 1589-1797 (see Table A-1);

and/or

iii) the CDR's may be CDR's that are derived by means of affinity maturation, for example starting from the CDR's of to the corresponding amino acid sequence of SEQ ID NO's: 1589-1797 (see Table A-1).

Preferably, the CDR sequences and FR sequences in the Nanobodies® of the invention are such that the Nanobodies® of the invention (and polypeptides of the invention comprising the same):

- bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles);

and/or such that they:

- bind to Dkk-1 with a k_{on}-rate of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$;

and/or such that they:

- bind to Dkk-1 with a k_{off} rate between 1 s^{-1} ($t_{1/2}$=0.69 s) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Preferably, CDR sequences and FR sequences present in the Nanobodies® of the invention are such that the Nanobodies® of the invention will bind to Dkk-1 with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

According to one non-limiting aspect of the invention, a Nanobody® may be as defined herein, but with the proviso that it has at least "one amino acid difference" (as defined herein) in at least one of the framework regions compared to the corresponding framework region of a naturally occurring human V_H domain, and in particular compared to the corresponding framework region of DP-47. More specifically, according to one non-limiting aspect of the invention, a Nanobody® may be as defined herein, but with the proviso that it has at least "one amino acid difference" (as defined herein) at at least one of the Hallmark residues (including those at positions 108, 103 and/or 45) compared to the corresponding framework region of a naturally occurring human V_H domain, and in particular compared to the corresponding framework region of DP-47. Usually, a Nanobody® will have at least one such amino acid difference with a naturally occurring V_H domain in at least one of FR2 and/or FR4, and in particular at at least one of the Hallmark residues in FR2 and/or FR4 (again, including those at positions 108, 103 and/or 45).

Also, a humanized Nanobody® of the invention may be as defined herein, but with the proviso that it has at least "one amino acid difference" (as defined herein) in at least one of the framework regions compared to the corresponding framework region of a naturally occurring V_{HH} domain. More specifically, according to one non-limiting aspect of the invention, a humanized Nanobody® may be as defined herein, but with the proviso that it has at least "one amino acid difference" (as defined herein) at at least one of the Hallmark residues (including those at positions

108, 103 and/or 45) compared to the corresponding framework region of a naturally occurring V_{HH} domain. Usually, a humanized Nanobody® will have at least one such amino acid difference with a naturally occurring V_{HH} domain in at least one of FR2 and/or FR4, and in particular at at least one of the Hallmark residues in FR2 and/or FR4 (again, including those at positions 108, 103 and/or 45).

As will be clear from the disclosure herein, it is also within the scope of the invention to use natural or synthetic analogs, mutants, variants, alleles, homologs and orthologs (herein collectively referred to as "*analogs*") of the Nanobodies® of the invention as defined herein, and in particular analogs of the Nanobodies® of SEQ ID NO's 1589-1797 (see Table A-1). Thus, according to one aspect of the invention, the term "Nanobody® of the invention" in its broadest sense also covers such analogs.

Generally, in such analogs, one or more amino acid residues may have been replaced, deleted and/or added, compared to the Nanobodies® of the invention as defined herein. Such substitutions, insertions or deletions may be made in one or more of the framework regions and/or in one or more of the CDR's. When such substitutions, insertions or deletions are made in one or more of the framework regions, they may be made at one or more of the Hallmark residues and/or at one or more of the other positions in the framework residues, although substitutions, insertions or deletions at the Hallmark residues are generally less preferred (unless these are suitable humanizing substitutions as described herein).

By means of non-limiting examples, a substitution may for example be a conservative substitution (as described herein) and/or an amino acid residue may be replaced by another amino acid residue that naturally occurs at the same position in another V_{HH} domain (see Tables B-4 to B-7 for some non-limiting examples of such substitutions), although the invention is generally not limited thereto. Thus, any one or more substitutions, deletions or insertions, or any combination thereof, that either improve the properties of the Nanobody® of the invention or that at least do not detract too much from the desired properties or from the balance or combination of desired properties of the Nanobody® of the invention (i.e. to the extent that the Nanobody® is no longer suited for its intended use) are included within the scope of the invention. A skilled person will generally be able to determine and select suitable substitutions, deletions or insertions, or suitable combinations of thereof, based on the disclosure herein and optionally after a limited degree of routine experimentation, which may for example involve introducing a limited number of possible substitutions and determining their influence on the properties of the Nanobodies® thus obtained.

For example, and depending on the host organism used to express the Nanobody® or polypeptide of the invention, such deletions and/or substitutions may be designed in such a way that one or more sites for post-translational modification (such as one or more glycosylation sites) are removed, as will be within the ability of the person skilled in the art. Alternatively, substitutions or

insertions may be designed so as to introduce one or more sites for attachment of functional groups (as described herein), for example to allow site-specific pegylation (again as described herein).

As can be seen from the data on the V_{HH} entropy and V_{HH} variability given in Tables B-4 to B-7 above, some amino acid residues in the framework regions are more conserved than others. Generally, although the invention in its broadest sense is not limited thereto, any substitutions, deletions or insertions are preferably made at positions that are less conserved. Also, generally, amino acid substitutions are preferred over amino acid deletions or insertions.

The analogs are preferably such that they can bind to Dkk-1 with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein for the Nanobodies® of the invention.

The analogs are preferably also such that they retain the favourable properties the Nanobodies®, as described herein.

Also, according to one preferred aspect, the analogs have a degree of sequence identity of at least 70%, preferably at least 80%, more preferably at least 90%, such as at least 95% or 99% or more; and/or preferably have at most 20, preferably at most 10, even more preferably at most 5, such as 4, 3, 2 or only 1 amino acid difference (as defined herein), with one of the Nanobodies® of SEQ ID NOs: 1589-1797 (see Table A-1).

Also, the framework sequences and CDR's of the analogs are preferably such that they are in accordance with the preferred aspects defined herein. More generally, as described herein, the analogs will have (a) a Q at position 108; and/or (b) a charged amino acid or a cysteine residue at position 45 and preferably an E at position 44, and more preferably E at position 44 and R at position 45; and/or (c) P, R or S at position 103.

One preferred class of analogs of the Nanobodies® of the invention comprise Nanobodies® that have been humanized (i.e. compared to the sequence of a naturally occurring Nanobody® of the invention). As mentioned in the background art cited herein, such humanization generally involves replacing one or more amino acid residues in the sequence of a naturally occurring V_{HH} with the amino acid residues that occur at the same position in a human V_H domain, such as a human V_H3 domain. Examples of possible humanizing substitutions or combinations of humanizing substitutions will be clear to the skilled person, for example from the Tables herein, from the possible humanizing substitutions mentioned in the background art cited herein, and/or from a comparison between the sequence of a Nanobody® and the sequence of a naturally occurring human V_H domain.

The humanizing substitutions should be chosen such that the resulting humanized Nanobodies® still retain the favourable properties of Nanobodies® as defined herein, and more preferably such that they are as described for analogs in the preceding paragraphs. A skilled person

will generally be able to determine and select suitable humanizing substitutions or suitable combinations of humanizing substitutions, based on the disclosure herein and optionally after a limited degree of routine experimentation, which may for example involve introducing a limited number of possible humanizing substitutions and determining their influence on the properties of the Nanobodies® thus obtained.

Generally, as a result of humanization, the Nanobodies® of the invention may become more "human-like", while still retaining the favorable properties of the Nanobodies® of the invention as described herein. As a result, such humanized Nanobodies® may have several advantages, such as a reduced immunogenicity, compared to the corresponding naturally occurring V_{HH} domains. Again, based on the disclosure herein and optionally after a limited degree of routine experimentation, the skilled person will be able to select humanizing substitutions or suitable combinations of humanizing substitutions which optimize or achieve a desired or suitable balance between the favourable properties provided by the humanizing substitutions on the one hand and the favourable properties of naturally occurring V_{HH} domains on the other hand.

The Nanobodies® of the invention may be suitably humanized at any framework residue(s), such as at one or more Hallmark residues (as defined herein) or at one or more other framework residues (i.e. non-Hallmark residues) or any suitable combination thereof. One preferred humanizing substitution for Nanobodies® of the "P,R,S-103 group" or the "KERE group" is Q108 into L108. Nanobodies® of the "GLEW class" may also be humanized by a Q108 into L108 substitution, provided at least one of the other Hallmark residues contains a camelid (camelizing) substitution (as defined herein). For example, as mentioned above, one particularly preferred class of humanized Nanobodies® has GLEW or a GLEW-like sequence at positions 44-47; P, R or S (and in particular R) at position 103, and an L at position 108.

The humanized and other analogs, and nucleic acid sequences encoding the same, can be provided in any manner known per se, for example using one or more of the techniques mentioned on pages 103 and 104 of WO 08/020079.

As mentioned there, it will be also be clear to the skilled person that the Nanobodies® of the invention (including their analogs) can be designed and/or prepared starting from human V_H sequences (i.e. amino acid sequences or the corresponding nucleotide sequences), such as for example from human V_H3 sequences such as DP-47, DP-51 or DP-29, i.e. by introducing one or more camelizing substitutions (i.e. changing one or more amino acid residues in the amino acid sequence of said human V_H domain into the amino acid residues that occur at the corresponding position in a V_{HH} domain), so as to provide the sequence of a Nanobody® of the invention and/or so as to confer the favourable properties of a Nanobody® to the sequence thus obtained. Again, this can generally

be performed using the various methods and techniques referred to in the previous paragraph, using an amino acid sequence and/or nucleotide sequence for a human V_H domain as a starting point.

Some preferred, but non-limiting camelizing substitutions can be derived from Tables B-4 – B-7. It will also be clear that camelizing substitutions at one or more of the Hallmark residues will generally have a greater influence on the desired properties than substitutions at one or more of the other amino acid positions, although both and any suitable combination thereof are included within the scope of the invention. For example, it is possible to introduce one or more camelizing substitutions that already confer at least some the desired properties, and then to introduce further camelizing substitutions that either further improve said properties and/or confer additional favourable properties. Again, the skilled person will generally be able to determine and select suitable camelizing substitutions or suitable combinations of camelizing substitutions, based on the disclosure herein and optionally after a limited degree of routine experimentation, which may for example involve introducing a limited number of possible camelizing substitutions and determining whether the favourable properties of Nanobodies® are obtained or improved (i.e. compared to the original V_H domain).

Generally, however, such camelizing substitutions are preferably such that the resulting an amino acid sequence at least contains (a) a Q at position 108; and/or (b) a charged amino acid or a cysteine residue at position 45 and preferably also an E at position 44, and more preferably E at position 44 and R at position 45; and/or (c) P, R or S at position 103; and optionally one or more further camelizing substitutions. More preferably, the camelizing substitutions are such that they result in a Nanobody® of the invention and/or in an analog thereof (as defined herein), such as in a humanized analog and/or preferably in an analog that is as defined in the preceding paragraphs.

Nanobodies® can also be derived from V_H domains by the incorporation of substitutions that are rare in nature, but nonetheless, structurally compatible with the VH domain fold. For example, but without being limiting, these substitutions may include on or more of the following: Gly at position 35, Ser, Val or Thr at position 37, Ser, Thr, Arg, Lys, His, Asp or Glu at position 39, Glu or His at position 45, Trp, Leu, Val, Ala, Thr, or Glu at position 47, S or R at position 50. (Barthelemy et al. J Biol Chem. 2008 Feb 8;283(6):3639-54. Epub 2007 Nov 28)

As will also be clear from the disclosure herein, it is also within the scope of the invention to use parts or fragments, or combinations of two or more parts or fragments, of the Nanobodies® of the invention as defined herein, and in particular parts or fragments of the Nanobodies® of SEQ ID NO's: 1589-1797 (see Table A-1). Thus, according to one aspect of the invention, the term "Nanobody® of the invention" in its broadest sense also covers such parts or fragments.

Generally, such parts or fragments of the Nanobodies® of the invention (including analogs thereof) have amino acid sequences in which, compared to the amino acid sequence of the

corresponding full length Nanobody® of the invention (or analog thereof), one or more of the amino acid residues at the N-terminal end, one or more amino acid residues at the C-terminal end, one or more contiguous internal amino acid residues, or any combination thereof, have been deleted and/or removed.

The parts or fragments are preferably such that they can bind to Dkk-1 with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein for the Nanobodies® of the invention.

Any part or fragment is preferably such that it comprises at least 10 contiguous amino acid residues, preferably at least 20 contiguous amino acid residues, more preferably at least 30 contiguous amino acid residues, such as at least 40 contiguous amino acid residues, of the amino acid sequence of the corresponding full length Nanobody® of the invention.

Also, any part or fragment is such preferably that it comprises at least one of CDR1, CDR2 and/or CDR3 or at least part thereof (and in particular at least CDR3 or at least part thereof). More preferably, any part or fragment is such that it comprises at least one of the CDR's (and preferably at least CDR3 or part thereof) and at least one other CDR (i.e. CDR1 or CDR2) or at least part thereof, preferably connected by suitable framework sequence(s) or at least part thereof. More preferably, any part or fragment is such that it comprises at least one of the CDR's (and preferably at least CDR3 or part thereof) and at least part of the two remaining CDR's, again preferably connected by suitable framework sequence(s) or at least part thereof.

According to another particularly preferred, but non-limiting aspect, such a part or fragment comprises at least CDR3, such as FR3, CDR3 and FR4 of the corresponding full length Nanobody® of the invention, i.e. as for example described in the International application WO 03/050531 (Lasters et al.).

As already mentioned above, it is also possible to combine two or more of such parts or fragments (i.e. from the same or different Nanobodies® of the invention), i.e. to provide an analog (as defined herein) and/or to provide further parts or fragments (as defined herein) of a Nanobody® of the invention. It is for example also possible to combine one or more parts or fragments of a Nanobody® of the invention with one or more parts or fragments of a human V_H domain.

According to one preferred aspect, the parts or fragments have a degree of sequence identity of at least 50%, preferably at least 60%, more preferably at least 70%, even more preferably at least 80%, such as at least 90%, 95% or 99% or more with one of the Nanobodies® of SEQ ID NOs 1589-1797 (see Table A-1).

The parts and fragments, and nucleic acid sequences encoding the same, can be provided and optionally combined in any manner known per se. For example, such parts or fragments can be

obtained by inserting a stop codon in a nucleic acid that encodes a full-sized Nanobody® of the invention, and then expressing the nucleic acid thus obtained in a manner known per se (e.g. as described herein). Alternatively, nucleic acids encoding such parts or fragments can be obtained by suitably restricting a nucleic acid that encodes a full-sized Nanobody® of the invention or by synthesizing such a nucleic acid in a manner known per se. Parts or fragments may also be provided using techniques for peptide synthesis known per se.

The invention in its broadest sense also comprises derivatives of the Nanobodies® of the invention. Such derivatives can generally be obtained by modification, and in particular by chemical and/or biological (e.g. enzymatical) modification, of the Nanobodies® of the invention and/or of one or more of the amino acid residues that form the Nanobodies® of the invention.

Examples of such modifications, as well as examples of amino acid residues within the Nanobody® sequence that can be modified in such a manner (i.e. either on the protein backbone but preferably on a side chain), methods and techniques that can be used to introduce such modifications and the potential uses and advantages of such modifications will be clear to the skilled person.

For example, such a modification may involve the introduction (e.g. by covalent linking or in an other suitable manner) of one or more functional groups, residues or moieties into or onto the Nanobody® of the invention, and in particular of one or more functional groups, residues or moieties that confer one or more desired properties or functionalities to the Nanobody® of the invention. Example of such functional groups will be clear to the skilled person.

For example, such modification may comprise the introduction (e.g. by covalent binding or in any other suitable manner) of one or more functional groups that increase the half-life, the solubility and/or the absorption of the Nanobody® of the invention, that reduce the immunogenicity and/or the toxicity of the Nanobody® of the invention, that eliminate or attenuate any undesirable side effects of the Nanobody® of the invention, and/or that confer other advantageous properties to and/or reduce the undesired properties of the Nanobodies® and/or polypeptides of the invention; or any combination of two or more of the foregoing. Examples of such functional groups and of techniques for introducing them will be clear to the skilled person, and can generally comprise all functional groups and techniques mentioned in the general background art cited hereinabove as well as the functional groups and techniques known per se for the modification of pharmaceutical proteins, and in particular for the modification of antibodies or antibody fragments (including ScFv's and single domain antibodies), for which reference is for example made to Remington's Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, PA (1980). Such functional groups may for example be linked directly (for example covalently) to a Nanobody® of the invention, or optionally via a suitable linker or spacer, as will again be clear to the skilled person.

One of the most widely used techniques for increasing the half-life and/or reducing the immunogenicity of pharmaceutical proteins comprises attachment of a suitable pharmacologically acceptable polymer, such as poly(ethyleneglycol) (PEG) or derivatives thereof (such as methoxypoly(ethylenegiycol) or mPEG). Generally, any suitable form of pegylation can be used, such as the pegylation used in the art for antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv's); reference is made to for example Chapman, Nat. Biotechnol., 54, 531-545 (2002); by Veronese and Harris, Adv. Drug Deliv. Rev. 54, 453-456 (2003), by Harris and Chess, Nat. Rev. Drug. Discov., 2, (2003) and in WO 04/060965. Various reagents for pegylation of proteins are also commercially available, for example from Nektar Therapeutics, USA.

Preferably, site-directed pegylation is used, in particular via a cysteine-residue (see for example Yang et al., Protein Engineering, 16, 10, 761-770 (2003). For example, for this purpose, PEG may be attached to a cysteine residue that naturally occurs in a Nanobody® of the invention, a Nanobody® of the invention may be modified so as to suitably introduce one or more cysteine residues for attachment of PEG, or an amino acid sequence comprising one or more cysteine residues for attachment of PEG may be fused to the N- and/or C-terminus of a Nanobody® of the invention, all using techniques of protein engineering known per se to the skilled person.

Preferably, for the Nanobodies® and proteins of the invention, a PEG is used with a molecular weight of more than 5000, such as more than 10,000 and less than 200,000, such as less than 100,000; for example in the range of 20,000-80,000.

Another, usually less preferred modification comprises N-linked or O-linked glycosylation, usually as part of co-translational and/or post-translational modification, depending on the host cell used for expressing the Nanobody® or polypeptide of the invention.

Yet another modification may comprise the introduction of one or more detectable labels or other signal-generating groups or moieties, depending on the intended use of the labelled Nanobody®. Suitable labels and techniques for attaching, using and detecting them will be clear to the skilled person, and for example include, but are not limited to, the fluorescent labels, phosphorescent labels, chemiluminescent labels, bioluminescent labels, radio-isotopes, metals, metal chelates, metallic cations, chromophores and enzymes, such as those mentioned on page 109 of WO 08/020079. Other suitable labels will be clear to the skilled person, and for example include moieties that can be detected using NMR or ESR spectroscopy.

Such labelled Nanobodies® and polypeptides of the invention may for example be used for in vitro, in vivo or in situ assays (including immunoassays known per se such as ELISA, RIA, EIA and other "sandwich assays", etc.) as well as in vivo diagnostic and imaging purposes, depending on the choice of the specific label.

As will be clear to the skilled person, another modification may involve the introduction of a chelating group, for example to chelate one of the metals or metallic cations referred to above. Suitable chelating groups for example include, without limitation, diethyl-enetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

Yet another modification may comprise the introduction of a functional group that is one part of a specific binding pair, such as the biotin-(strept)avidin binding pair. Such a functional group may be used to link the Nanobody® of the invention to another protein, polypeptide or chemical compound that is bound to the other half of the binding pair, i.e. through formation of the binding pair. For example, a Nanobody® of the invention may be conjugated to biotin, and linked to another protein, polypeptide, compound or carrier conjugated to avidin or streptavidin. For example, such a conjugated Nanobody® may be used as a reporter, for example in a diagnostic system where a detectable signal-producing agent is conjugated to avidin or streptavidin. Such binding pairs may for example also be used to bind the Nanobody® of the invention to a carrier, including carriers suitable for pharmaceutical purposes. One non-limiting example are the liposomal formulations described by Cao and Suresh, Journal of Drug Targetting, 8, 4, 257 (2000). Such binding pairs may also be used to link a therapeutically active agent to the Nanobody® of the invention.

For some applications, in particular for those applications in which it is intended to kill a cell that expresses the target against which the Nanobodies® of the invention are directed (e.g. in the treatment of cancer), or to reduce or slow the growth and/or proliferation such a cell, the Nanobodies® of the invention may also be linked to a toxin or to a toxic residue or moiety. Examples of toxic moieties, compounds or residues which can be linked to a Nanobody® of the invention to provide – for example – a cytotoxic compound will be clear to the skilled person and can for example be found in the prior art cited above and/or in the further description herein. One example is the so-called ADEPT™ technology described in WO 03/055527.

Other potential chemical and enzymatical modifications will be clear to the skilled person. Such modifications may also be introduced for research purposes (e.g. to study function-activity relationships). Reference is for example made to Lundblad and Bradshaw, Biotechnol. Appl. Biochem., 26, 143-151 (1997).

Preferably, the derivatives are such that they bind to Dkk-1 with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein for the Nanobodies® of the invention.

As mentioned above, the invention also relates to proteins or polypeptides that essentially consist of or comprise at least one Nanobody® of the invention. By "essentially consist of" is meant that the amino acid sequence of the polypeptide of the invention either is exactly the same as the

amino acid sequence of a Nanobody® of the invention or corresponds to the amino acid sequence of a Nanobody® of the invention which has a limited number of amino acid residues, such as 1-20 amino acid residues, for example 1-10 amino acid residues and preferably 1-6 amino acid residues, such as 1, 2, 3, 4, 5 or 6 amino acid residues, added at the amino terminal end, at the carboxy terminal end, or at both the amino terminal end and the carboxy terminal end of the amino acid sequence of the Nanobody®.

Said amino acid residues may or may not change, alter or otherwise influence the (biological) properties of the Nanobody® and may or may not add further functionality to the Nanobody®. For example, such amino acid residues:

- can comprise an N-terminal Met residue, for example as result of expression in a heterologous host cell or host organism.
- may form a signal sequence or leader sequence that directs secretion of the Nanobody® from a host cell upon synthesis. Suitable secretory leader peptides will be clear to the skilled person, and may be as further described herein. Usually, such a leader sequence will be linked to the N-terminus of the Nanobody®, although the invention in its broadest sense is not limited thereto;
- may form a sequence or signal that allows the Nanobody® to be directed towards and/or to penetrate or enter into specific organs, tissues, cells, or parts or compartments of cells, and/or that allows the Nanobody® to penetrate or cross a biological barrier such as a cell membrane, a cell layer such as a layer of epithelial cells, a tumor including solid tumors, or the blood-brain-barrier. Examples of such amino acid sequences will be clear to the skilled person and include those mentioned in paragraph c) on page 112 of WO 08/020079.
- may form a "tag", for example an amino acid sequence or residue that allows or facilitates the purification of the Nanobody®, for example using affinity techniques directed against said sequence or residue. Thereafter, said sequence or residue may be removed (e.g. by chemical or enzymatical cleavage) to provide the Nanobody® sequence (for this purpose, the tag may optionally be linked to the Nanobody® sequence via a cleavable linker sequence or contain a cleavable motif). Some preferred, but non-limiting examples of such residues are multiple histidine residues, glutatione residues and a myc-tag (see for example SEQ ID NO:31 of WO 06/12282).
- may be one or more amino acid residues that have been functionalized and/or that can serve as a site for attachment of functional groups. Suitable amino acid residues and functional groups will be clear to the skilled person and include, but are not limited to, the amino acid residues and functional groups mentioned herein for the derivatives of the Nanobodies® of the invention.

According to another aspect, a polypeptide of the invention comprises a Nanobody® of the invention, which is fused at its amino terminal end, at its carboxy terminal end, or both at its amino terminal end and at its carboxy terminal end to at least one further amino acid sequence, i.e. so as to provide a fusion protein comprising said Nanobody® of the invention and the one or more further amino acid sequences. Such a fusion will also be referred to herein as a "Nanobody® fusion".

The one or more further amino acid sequence may be any suitable and/or desired amino acid sequences. The further amino acid sequences may or may not change, alter or otherwise influence the (biological) properties of the Nanobody®, and may or may not add further functionality to the Nanobody® or the polypeptide of the invention. Preferably, the further amino acid sequence is such that it confers one or more desired properties or functionalities to the Nanobody® or the polypeptide of the invention.

For example, the further amino acid sequence may also provide a second binding site, which binding site may be directed against any desired protein, polypeptide, antigen, antigenic determinant or epitope (including but not limited to the same protein, polypeptide, antigen, antigenic determinant or epitope against which the Nanobody® of the invention is directed, or a different protein, polypeptide, antigen, antigenic determinant or epitope).

Example of such amino acid sequences will be clear to the skilled person, and may generally comprise all amino acid sequences that are used in peptide fusions based on conventional antibodies and fragments thereof (including but not limited to ScFv's and single domain antibodies). Reference is for example made to the review by Holliger and Hudson, Nature Biotechnology, 23, 9, 1126-1136 (2005).

For example, such an amino acid sequence may be an amino acid sequence that increases the half-life, the solubility, or the absorption, reduces the immunogenicity or the toxicity, eliminates or attenuates undesirable side effects, and/or confers other advantageous properties to and/or reduces the undesired properties of the polypeptides of the invention, compared to the Nanobody® of the invention per se. Some non-limiting examples of such amino acid sequences are serum proteins, such as human serum albumin (see for example WO 00/27435) or haptenic molecules (for example haptens that are recognized by circulating antibodies, see for example WO 98/22141).

In particular, it has been described in the art that linking fragments of immunoglobulins (such as V_H domains) to serum albumin or to fragments thereof can be used to increase the half-life. Reference is for made to WO 00/27435 and WO 01/077137). According to the invention, the Nanobody® of the invention is preferably either directly linked to serum albumin (or to a suitable fragment thereof) or via a suitable linker, and in particular via a suitable peptide linked so that the polypeptide of the invention can be expressed as a genetic fusion (protein). According to one specific aspect, the Nanobody® of the invention may be linked to a fragment of serum albumin that at least

comprises the domain III of serum albumin or part thereof. Reference is for example made to WO 07/112940 of Ablynx N.V.

Alternatively, the further amino acid sequence may provide a second binding site or binding unit that is directed against a serum protein (such as, for example, human serum albumin or another serum protein such as IgG), so as to provide increased half-life in serum. Such amino acid sequences for example include the Nanobodies® described below, as well as the small peptides and binding proteins described in WO 91/01743, WO 01/45746 and WO 02/076489 and the dAb's described in WO 03/002609 and WO 04/003019. Reference is also made to Harmsen et al., Vaccine, 23 (41); 4926-42, 2005, as well as to EP 0 368 684, as well as to WO 08/028977, WO 08/043821, WO 08/043822 by Ablynx N.V. and US provisional application of Ablynx N.V. entitled *"Peptides capable of binding to serum proteins"* filed on December 5, 2006 ((see also PCT/EP2007/063348).

Such amino acid sequences may in particular be directed against serum albumin (and more in particular human serum albumin) and/or against IgG (and more in particular human IgG). For example, such amino acid sequences may be amino acid sequences that are directed against (human) serum albumin and amino acid sequences that can bind to amino acid residues on (human) serum albumin that are not involved in binding of serum albumin to FcRn (see for example WO 06/0122787) and/or amino acid sequences that are capable of binding to amino acid residues on serum albumin that do not form part of domain III of serum albumin (see again for example WO 06/0122787); amino acid sequences that have or can provide an increased half-life (see for example WO 08/028977 by Ablynx N.V.); amino acid sequences against human serum albumin that are cross-reactive with serum albumin from at least one species of mammal, and in particular with at least one species of primate (such as, without limitation, monkeys from the genus *Macaca* (such as, and in particular, cynomologus monkeys (*Macaca fascicularis*) and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*), reference is again made to WO 08/028977; amino acid sequences that can bind to serum albumin in a pH independent manner (see for example WO 08/043821by Ablynx N.V. entitled *"Amino acid sequences that bind to serum proteins in a manner that is essentially independent of the pH, compounds comprising the same, and uses thereof"*) and/or amino acid sequences that are conditional binders (see for example WO 08/043822 by Ablynx N.V. entitled *"Amino acid sequences that bind to a desired molecule in a conditional manner"*).

According to another aspect, the one or more further amino acid sequences may comprise one or more parts, fragments or domains of conventional 4-chain antibodies (and in particular human antibodies) and/or of heavy chain antibodies. For example, although usually less preferred, a Nanobody® of the invention may be linked to a conventional (preferably human) V_H or V_L domain or to a natural or synthetic analog of a V_H or V_L domain, again optionally via a linker sequence (including but not limited to other (single) domain antibodies, such as the dAb's described by Ward et al.).

The at least one Nanobody® may also be linked to one or more (preferably human) C_H1, C_H2 and/or C_H3 domains, optionally via a linker sequence. For instance, a Nanobody® linked to a suitable C_H1 domain could for example be used - together with suitable light chains - to generate antibody fragments/structures analogous to conventional Fab fragments or $F(ab')_2$ fragments, but in which one or (in case of an $F(ab')_2$ fragment) one or both of the conventional V_H domains have been replaced by a Nanobody® of the invention. Also, two Nanobodies® could be linked to a C_H3 domain (optionally via a linker) to provide a construct with increased half-life in vivo.

According to one specific aspect of a polypeptide of the invention, one or more Nanobodies® of the invention may be linked (optionally via a suitable linker or hinge region) to one or more constant domains (for example, 2 or 3 constant domains that can be used as part of/to form an Fc portion), to an Fc portion and/or to one or more antibody parts, fragments or domains that confer one or more effector functions to the polypeptide of the invention and/or may confer the ability to bind to one or more Fc receptors. For example, for this purpose, and without being limited thereto, the one or more further amino acid sequences may comprise one or more C_H2 and/or C_H3 domains of an antibody, such as from a heavy chain antibody (as described herein) and more preferably from a conventional human 4-chain antibody; and/or may form (part of) and Fc region, for example from IgG (e.g. from IgG1, IgG2, IgG3 or IgG4), from IgE or from another human Ig such as IgA, IgD or IgM. For example, WO 94/04678 describes heavy chain antibodies comprising a Camelid V_{HH} domain or a humanized derivative thereof (i.e. a Nanobody®), in which the Camelidae C_H2 and/or C_H3 domain have been replaced by human C_H2 and C_H3 domains, so as to provide an immunoglobulin that consists of 2 heavy chains each comprising a Nanobody® and human C_H2 and C_H3 domains (but no C_H1 domain), which immunoglobulin has the effector function provided by the C_H2 and C_H3 domains and which immunoglobulin can function without the presence of any light chains. Other amino acid sequences that can be suitably linked to the Nanobodies® of the invention so as to provide an effector function will be clear to the skilled person, and may be chosen on the basis of the desired effector function(s). Reference is for example made to WO 04/058820, WO 99/42077, WO 02/056910 and WO 05/017148, as well as the review by Holliger and Hudson, supra; and to the non-prepublished US provisional application by Ablynx N.V. entitled *"Constructs comprising single variable domains and an Fc portion derived from IgE"* which has a filing date of December 4, 2007. Coupling of a Nanobody® of the invention to an Fc portion may also lead to an increased half-life, compared to the corresponding Nanobody® of the invention. For some applications, the use of an Fc portion and/or of constant domains (i.e. C_H2 and/or C_H3 domains) that confer increased half-life without any biologically significant effector function may also be suitable or even preferred. Other suitable constructs comprising one or more Nanobodies® and one or more constant domains with increased half-life in vivo will be clear to the skilled person, and may for example comprise two Nanobodies®

linked to a C_H3 domain, optionally via a linker sequence. Generally, any fusion protein or derivatives with increased half-life will preferably have a molecular weight of more than 50 kD, the cut-off value for renal absorption.

In another one specific, but non-limiting, aspect, in order to form a polypeptide of the invention, one or more amino acid sequences of the invention may be linked (optionally via a suitable linker or hinge region) to naturally occurring, synthetic or semisynthetic constant domains (or analogs, variants, mutants, parts or fragments thereof) that have a reduced (or essentially no) tendency to self-associate into dimers (i.e. compared to constant domains that naturally occur in conventional 4-chain antibodies). Such monomeric (i.e. not self-associating) Fc chain variants, or fragments thereof, will be clear to the skilled person. For example, Helm et al., J Biol Chem 1996 271 7494, describe monomeric Fc chain variants that can be used in the polypeptide chains of the invention.

Also, such monomeric Fc chain variants are preferably such that they are still capable of binding to the complement or the relevant Fc receptor(s) (depending on the Fc portion from which they are derived), and/or such that they still have some or all of the effector functions of the Fc portion from which they are derived (or at a reduced level still suitable for the intended use). Alternatively, in such a polypeptide chain of the invention, the monomeric Fc chain may be used to confer increased half-life upon the polypeptide chain, in which case the monomeric Fc chain may also have no or essentially no effector functions.

Bivalent/multivalent, bispecific/multispecific or biparatopic/multiparatopic polypeptides of the invention may also be linked to Fc portions, in order to provide polypeptide constructs of the type that is described in the non-prepublished US provisional application US 61/005,331 entitled *"immunoglobulin constructs"* filed on December 4, 2007.

The further amino acid sequences may also form a signal sequence or leader sequence that directs secretion of the Nanobody® or the polypeptide of the invention from a host cell upon synthesis (for example to provide a pre-, pro- or prepro- form of the polypeptide of the invention, depending on the host cell used to express the polypeptide of the invention).

The further amino acid sequence may also form a sequence or signal that allows the Nanobody® or polypeptide of the invention to be directed towards and/or to penetrate or enter into specific organs, tissues, cells, or parts or compartments of cells, and/or that allows the Nanobody® or polypeptide of the invention to penetrate or cross a biological barrier such as a cell membrane, a cell layer such as a layer of epithelial cells, a tumor including solid tumors, or the blood-brain-barrier. Suitable examples of such amino acid sequences will be clear to the skilled person, and for example include, but are not limited to, those mentioned on page 118 of WO 08/020079. For some applications, in particular for those applications in which it is intended to kill a cell that expresses the

target against which the Nanobodies® of the invention are directed (e.g. in the treatment of cancer), or to reduce or slow the growth and/or proliferation of such a cell, the Nanobodies® of the invention may also be linked to a (cyto)toxic protein or polypeptide. Examples of such toxic proteins and polypeptides which can be linked to a Nanobody® of the invention to provide – for example – a cytotoxic polypeptide of the invention will be clear to the skilled person and can for example be found in the prior art cited above and/or in the further description herein. One example is the so-called ADEPT™ technology described in WO 03/055527.

According to one preferred, but non-limiting aspect, said one or more further amino acid sequences comprise at least one further Nanobody®, so as to provide a polypeptide of the invention that comprises at least two, such as three, four, five or more Nanobodies®, in which said Nanobodies® may optionally be linked via one or more linker sequences (as defined herein). As described on pages 119 and 120 of WO 08/020079, polypeptides of the invention that comprise two or more Nanobodies®, of which at least one is a Nanobody® of the invention, will also be referred to herein as "multivalent" polypeptides of the invention, and the Nanobodies® present in such polypeptides will also be referred to herein as being in a "multivalent format". For example, "bivalent" and "trivalent" polypeptides of the invention may be as further described on pages 119 and 120 of WO 08/020079.

Polypeptides of the invention that contain at least two Nanobodies®, in which at least one Nanobody® is directed against a first antigenic determinant on Dkk-1 and at least one Nanobody® is directed against a second antigenic determinant on Dkk-1 will also be referred to as "multiparatopic" polypeptides of the invention, and the Nanobodies® present in such polypeptides will also be referred to herein as being in a "multiparatopic format". Thus, for example, a "biparatopic" polypeptide of the invention is a polypeptide that comprises at least one Nanobody® directed against a first antigenic determinant on Dkk-1 and at least one further Nanobody® directed against a second antigenic determinant on Dkk-1, whereas a "triparatopic" polypeptide of the invention is a polypeptide that comprises at least one Nanobody® directed against a first antigenic determinant on Dkk-1, at least one further Nanobody® directed against a second antigenic determinant on Dkk-1 and at least one further Nanobody® directed against a third antigenic determinant on Dkk-1; etc.

Accordingly, in its simplest form, a biparatopic polypeptide of the invention is a bivalent polypeptide of the invention (as defined herein), comprising a first Nanobody® directed against a first antigenic determinant on Dkk-1, and a second Nanobody® directed against a second antigenic determinant on Dkk-1, in which said first and second Nanobody® may optionally be linked via a linker sequence (as defined herein); whereas a triparatopic polypeptide of the invention in its simplest form is a trivalent polypeptide of the invention (as defined herein), comprising a first Nanobody® directed against a first antigenic determinant on Dkk-1, a second Nanobody® directed against a second

antigenic determinant on Dkk-1 and a third Nanobody® directed against a third antigenic determinant on Dkk-1, in which said first, second and third Nanobody® may optionally be linked via one or more, and in particular one and more, in particular two, linker sequences.

However, as will be clear from the description hereinabove, the invention is not limited thereto, in the sense that a multiparatopic polypeptide of the invention may comprise at least one Nanobody® against a first antigenic determinant on Dkk-1, and any number of Nanobodies® directed against one or more other antigenic determinants on Dkk-1.

Polypeptides of the invention that contain at least two Nanobodies®, in which at least one Nanobody® is directed against a first antigen (i.e. against Dkk-1],) and at least one Nanobody® is directed against a second antigen (i.e. different from Dkk-1), will also be referred to as "multispecific" polypeptides of the invention, and the Nanobodies® present in such polypeptides will also be referred to herein as being in a "multispecific format". Thus, for example, a "bispecific" polypeptide of the invention is a polypeptide that comprises at least one Nanobody® directed against a first antigen (i.e. Dkk-1) and at least one further Nanobody® directed against a second antigen (i.e. different from Dkk-1), whereas a "trispecific" polypeptide of the invention is a polypeptide that comprises at least one Nanobody® directed against a first antigen (i.e. Dkk-1), at least one further Nanobody® directed against a second antigen (i.e. different from Dkk-1) and at least one further Nanobody® directed against a third antigen (i.e. different from both Dkk-1, and the second antigen); etc.

Accordingly, in its simplest form, a bispecific polypeptide of the invention is a bivalent polypeptide of the invention (as defined herein), comprising a first Nanobody® directed against Dkk-1, and a second Nanobody® directed against a second antigen, in which said first and second Nanobody® may optionally be linked via a linker sequence (as defined herein); whereas a trispecific polypeptide of the invention in its simplest form is a trivalent polypeptide of the invention (as defined herein), comprising a first Nanobody® directed against Dkk-1, a second Nanobody® directed against a second antigen and a third Nanobody® directed against a third antigen, in which said first, second and third Nanobody® may optionally be linked via one or more, and in particular one and more, in particular two, linker sequences.

However, as will be clear from the description hereinabove, the invention is not limited thereto, in the sense that a multispecific polypeptide of the invention may comprise at least one Nanobody® against Dkk-1, and any number of Nanobodies® directed against one or more antigens different from Dkk-1.

Furthermore, although it is encompassed within the scope of the invention that the specific order or arrangement of the various Nanobodies® in the polypeptides of the invention may have some influence on the properties of the final polypeptide of the invention (including but not limited

to the affinity, specificity or avidity for Dkk-1, or against the one or more other antigens), said order or arrangement is usually not critical and may be suitably chosen by the skilled person, optionally after some limited routine experiments based on the disclosure herein. Thus, when reference is made to a specific multivalent or multispecific polypeptide of the invention, it should be noted that this encompasses any order or arrangements of the relevant Nanobodies®, unless explicitly indicated otherwise.

Finally, it is also within the scope of the invention that the polypeptides of the invention contain two or more Nanobodies® and one or more further amino acid sequences (as mentioned herein).

For multivalent and multispecific polypeptides containing one or more V_{HH} domains and their preparation, reference is also made to Conrath et al., J. Biol. Chem., Vol. 276, 10. 7346-7350, 2001; Muyldermans, Reviews in Molecular Biotechnology 74 (2001), 277-302; as well as to for example WO 96/34103 and WO 99/23221. Some other examples of some specific multispecific and/or multivalent polypeptide of the invention can be found in the applications by Ablynx N.V. referred to herein.

One preferred, but non-limiting example of a multispecific polypeptide of the invention comprises at least one Nanobody® of the invention and at least one Nanobody® that provides for an increased half-life. Such Nanobodies® may for example be Nanobodies® that are directed against a serum protein, and in particular a human serum protein, such as human serum albumin, thyroxine-binding protein, (human) transferrin, fibrinogen, an immunoglobulin such as IgG, IgE or IgM, or against one of the serum proteins listed in WO 04/003019. Of these, Nanobodies® that can bind to serum albumin (and in particular human serum albumin) or to IgG (and in particular human IgG, see for example Nanobody® VH-1 described in the review by Muyldermans, *supra*) are particularly preferred (although for example, for experiments in mice or primates, Nanobodies® against or cross-reactive with mouse serum albumin (MSA) or serum albumin from said primate, respectively, can be used. However, for pharmaceutical use, Nanobodies® against human serum albumin or human IgG will usually be preferred). Nanobodies® that provide for increased half-life and that can be used in the polypeptides of the invention include the Nanobodies® directed against serum albumin that are described in WO 04/041865, in WO 06/122787 and in the further patent applications by Ablynx N.V., such as those mentioned above.

For example, the some preferred Nanobodies® that provide for increased half-life for use in the present invention include Nanobodies® that can bind to amino acid residues on (human) serum albumin that are not involved in binding of serum albumin to FcRn (see for example WO 06/0122787); Nanobodies® that are capable of binding to amino acid residues on serum albumin that do not form part of domain III of serum albumin (see for example WO 06/0122787); Nanobodies® that have or can provide an increased half-life (see for example WO 08/028977 by Ablynx N.V

mentioned herein); Nanobodies® against human serum albumin that are cross-reactive with serum albumin from at least one species of mammal, and in particular with at least one species of primate (such as, without limitation, monkeys from the genus *Macaca* (such as, and in particular, cynomologus monkeys (*Macaca fascicularis*) and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*)) (see for example WO 08/028977 by Ablynx N.V)); Nanobodies® that can bind to serum albumin in a pH independent manner (see for example WO2008/043821 by Ablynx N.V. mentioned herein) and/or Nanobodies® that are conditional binders (see for example WO 08/043822by Ablynx N.V.).

Some particularly preferred Nanobodies® that provide for increased half-life and that can be used in the polypeptides of the invention include the Nanobodies® ALB-1 to ALB-10 disclosed in WO 06/122787 (see Tables II and III) of which ALB-8 (SEQ ID NO: 62 in WO 06/122787) is particularly preferred.

Some preferred, but non-limiting examples of polypeptides of the invention that comprise at least one Nanobody® of the invention and at least one Nanobody® that provides for increased half-life are given in SEQ ID NO's 1817-1820.

According to a specific, but non-limiting aspect of the invention, the polypeptides of the invention contain, besides the one or more Nanobodies® of the invention, at least one Nanobody® against human serum albumin.

Generally, any polypeptides of the invention with increased half-life that contain one or more Nanobodies® of the invention, and any derivatives of Nanobodies® of the invention or of such polypeptides that have an increased half-life, preferably have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding Nanobody® of the invention per se. For example, such a derivative or polypeptides with increased half-life may have a half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding Nanobody® of the invention per se.

In a preferred, but non-limiting aspect of the invention, such derivatives or polypeptides may exhibit a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more. For example, such derivatives or polypeptides may have a half-life of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

According to one aspect of the invention the polypeptides are capable of binding to one or more molecules which can increase the half-life of the polypeptide in vivo.

The polypeptides of the invention are stabilised in vivo and their half-life increased by binding to molecules which resist degradation and/or clearance or sequestration. Typically, such molecules are naturally occurring proteins which themselves have a long half-life in vivo.

Another preferred, but non-limiting example of a multispecific polypeptide of the invention comprises at least one Nanobody® of the invention and at least one Nanobody® that directs the polypeptide of the invention towards, and/or that allows the polypeptide of the invention to penetrate or to enter into specific organs, tissues, cells, or parts or compartments of cells, and/or that allows the Nanobody® to penetrate or cross a biological barrier such as a cell membrane, a cell layer such as a layer of epithelial cells, a tumor including solid tumors, or the blood-brain-barrier. Examples of such Nanobodies® include Nanobodies® that are directed towards specific cell-surface proteins, markers or epitopes of the desired organ, tissue or cell (for example cell-surface markers associated with tumor cells), and the single-domain brain targeting antibody fragments described in WO 02/057445 and WO 06/040153, of which FC44 (SEQ ID NO: 189 of WO 06/040153) and FC5 (SEQ ID NO: 190 of WO 06/040154) are preferred examples.

In the polypeptides of the invention, the one or more Nanobodies® and the one or more polypeptides may be directly linked to each other (as for example described in WO 99/23221) and/or may be linked to each other via one or more suitable spacers or linkers, or any combination thereof.

Suitable spacers or linkers for use in multivalent and multispecific polypeptides will be clear to the skilled person, and may generally be any linker or spacer used in the art to link amino acid sequences. Preferably, said linker or spacer is suitable for use in constructing proteins or polypeptides that are intended for pharmaceutical use.

Some particularly preferred spacers include the spacers and linkers that are used in the art to link antibody fragments or antibody domains. These include the linkers mentioned in the general background art cited above, as well as for example linkers that are used in the art to construct diabodies or ScFv fragments (in this respect, however, its should be noted that, whereas in diabodies and in ScFv fragments, the linker sequence used should have a length, a degree of flexibility and other properties that allow the pertinent V_H and V_L domains to come together to form the complete antigen-binding site, there is no particular limitation on the length or the flexibility of the linker used in the polypeptide of the invention, since each Nanobody® by itself forms a complete antigen-binding site).

For example, a linker may be a suitable amino acid sequence, and in particular amino acid sequences of between 1 and 50, preferably between 1 and 30, such as between 1 and 10 amino acid residues. Some preferred examples of such amino acid sequences include gly-ser linkers, for example

of the type $(gly_xser_y)_z$, such as (for example $(gly_4ser)_3$ or $(gly_3ser_2)_3$, as described in WO 99/42077 and the GS30, GS15, GS9 and GS7 linkers described in the applications by Ablynx mentioned herein (see for example WO 06/040153 and WO 06/122825), as well as hinge-like regions, such as the hinge regions of naturally occurring heavy chain antibodies or similar sequences (such as described in WO 94/04678).

Some other particularly preferred linkers are poly-alanine (such as AAA), as well as the linkers GS30 (SEQ ID NO: 85 in WO 06/122825) and GS9 (SEQ ID NO: 84 in WO 06/122825).

Other suitable linkers generally comprise organic compounds or polymers, in particular those suitable for use in proteins for pharmaceutical use. For instance, poly(ethyleneglycol) moieties have been used to link antibody domains, see for example WO 04/081026.

It is encompassed within the scope of the invention that the length, the degree of flexibility and/or other properties of the linker(s) used (although not critical, as it usually is for linkers used in ScFv fragments) may have some influence on the properties of the final polypeptide of the invention, including but not limited to the affinity, specificity or avidity for Dkk-1, or for one or more of the other antigens. Based on the disclosure herein, the skilled person will be able to determine the optimal linker(s) for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

For example, in multivalent polypeptides of the invention that comprise Nanobodies® directed against a multimeric antigen (such as a multimeric receptor or other protein), the length and flexibility of the linker are preferably such that it allows each Nanobody® of the invention present in the polypeptide to bind to the antigenic determinant on each of the subunits of the multimer. Similarly, in a multispecific polypeptide of the invention that comprises Nanobodies® directed against two or more different antigenic determinants on the same antigen (for example against different epitopes of an antigen and/or against different subunits of a multimeric receptor, channel or protein), the length and flexibility of the linker are preferably such that it allows each Nanobody® to bind to its intended antigenic determinant. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linker(s) for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

For example, as further described herein, some of the most preferred multiparatopic polypeptides of the invention comprise (i) at least one amino acid sequence of the invention (and in particular at least one Nanobody®) that is directed against the LRP5/6 binding site on Dkk-1 (and in particular against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 on Dkk-1, preferably against at least one of amino acid residues R242 or H210 on Dkk-1) and/or that is capable of competing with LRP5/6 for binding to Dkk-1; and at least one amino acid sequence of the invention (and in particular at least one Nanobody®) that is directed against the Kremen binding

site on Dkk-1 (and in particular against at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably against at least one of amino acid residues R197 or K232 on Dkk-1) and/or that is capable of competing with Kremen for binding to Dkk-1. In such a preferred multiparatopic polypeptide of the invention, the linker is most preferably such that the multiparatopic polypeptide of the invention is capable of (simultaneously) binding to both the LRP5/6 binding site on Dkk-1 (and in particular to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 on Dkk-1, preferably to at least one of amino acid residues R242 or H210 on Dkk-1) as well as the Kremen binding site on Dkk-1 (and in particular to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1, preferably to at least one of amino acid residues R197 or K232 on Dkk-1), again most preferably so as to allow binding with increased avidity and also intramolecular binding and/or recognition. Such multiparatopic polypeptides of the invention with such a linker form a particularly preferred aspect of the invention, and examples of such a linker are given in the Examples below. For example, when such a linker is a Gly-Ser linker (for example, a Gly-Ser linker as described in the Examples), it preferably has a length of at least 15 amino acid residues, such as at least 20 or at least 30 amino acid residues. The maximum length is not especially critical, but for practical considerations (such as ease of cloning and expression) the linker is preferably no longer than 75 amino acid residues, more preferably less than 50 amino acid residues. For example, Gly-Ser linkers (such as the Gly-Ser linkers as described in the Examples) of between 20 and 40 amino acid residues, such as about 25, 30 or 35 amino acid residues, may be particularly suited. Based on the disclosure herein, the skilled person will be able to determine other suitable linkers, it being understood that the optimal length of each linker may also depend on the amino acid composition of the linker that is envisaged for use.

Optimal linker lengths in biparatopic, triparatopic or multiparatopic polypeptides of the invention can, for example, be designed in silico with any method for protein design known in the art or disclosed herein (see, e.g. the Example section). Optimal linker lengths, for example obtained by in silico design, can further be verified experimentally by binding and competition assays as will be known to the skilled person and/or described herein (see e.g. the example section). Optimal linker lengths in biparatopic, triparatopic or multiparatopic polypeptides may also be determined using the screening method for determining optimal linker length as described herein.

The choice of the format (N – or C-terminal position of the different Nanobodies®) of the biparatopic, triparatopic or multiparatopic polypeptides of the invention and linker length can also be used to obtain molecules that bind avidly to the target antigen (via two, or more, binding sites), yet are purposely not agonistic. By optimising the format and linker length and composition, the binding sites can be positioned in such way that simultaneous binding of two or more Nanobodies® to the same target antigen (i.e. intramolecular binding) will be highly favoured compared to binding to

separate antigens in proximity of one another (intermolecular binding, such as e.g. on a cell surface). This could, for example, reduce the chance on agonism (which might not be desired in a good therapeutic compound). Screening and/or selection methods and assays are known to the skilled person and/or described herein that allow for the isolation of avidity binding domains positioned in relation to one another and to the antigen of interest in such way as to have an antagonistic function only.

It is also within the scope of the invention that the linker(s) used confer one or more other favourable properties or functionality to the polypeptides of the invention, and/or provide one or more sites for the formation of derivatives and/or for the attachment of functional groups (e.g. as described herein for the derivatives of the Nanobodies® of the invention). For example, linkers containing one or more charged amino acid residues (see Table A-2 on page 48 of the International application WO 08/020079) can provide improved hydrophilic properties, whereas linkers that form or contain small epitopes or tags can be used for the purposes of detection, identification and/or purification. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linkers for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

Finally, when two or more linkers are used in the polypeptides of the invention, these linkers may be the same or different. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linkers for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

Usually, for easy of expression and production, a polypeptide of the invention will be a linear polypeptide. However, the invention in its broadest sense is not limited thereto. For example, when a polypeptide of the invention comprises three of more Nanobodies®, it is possible to link them by use of a linker with three or more "arms", which each "arm" being linked to a Nanobody®, so as to provide a "star-shaped" construct. It is also possible, although usually less preferred, to use circular constructs.

The invention also comprises derivatives of the polypeptides of the invention, which may be essentially analogous to the derivatives of the Nanobodies® of the invention, i.e. as described herein.

The invention also comprises proteins or polypeptides that "essentially consist" of a polypeptide of the invention (in which the wording "essentially consist of" has essentially the same meaning as indicated hereinabove).

According to one aspect of the invention, the polypeptide of the invention is in essentially isolated from, as defined herein.

The amino acid sequences, Nanobodies®, polypeptides and nucleic acids of the invention can be prepared in a manner known per se, as will be clear to the skilled person from the further

description herein. For example, the Nanobodies® and polypeptides of the invention can be prepared in any manner known per se for the preparation of antibodies and in particular for the preparation of antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments). Some preferred, but non-limiting methods for preparing the amino acid sequences, Nanobodies®, polypeptides and nucleic acids include the methods and techniques described herein.

As will be clear to the skilled person, one particularly useful method for preparing an amino acid sequence, Nanobody® and/or a polypeptide of the invention generally comprises the steps of:

i) the expression, in a suitable host cell or host organism (also referred to herein as a "host of the invention") or in another suitable expression system of a nucleic acid that encodes said amino acid sequence, Nanobody® or polypeptide of the invention (also referred to herein as a "*nucleic acid of the invention*"), optionally followed by:

ii) isolating and/or purifying the amino acid sequence, Nanobody® or polypeptide of the invention thus obtained.

In particular, such a method may comprise the steps of:

i) cultivating and/or maintaining a host of the invention under conditions that are such that said host of the invention expresses and/or produces at least one amino acid sequence, Nanobody® and/or polypeptide of the invention; optionally followed by:

ii) isolating and/or purifying the amino acid sequence, Nanobody® or polypeptide of the invention thus obtained.

A nucleic acid of the invention can be in the form of single or double stranded DNA or RNA, and is preferably in the form of double stranded DNA. For example, the nucleotide sequences of the invention may be genomic DNA, cDNA or synthetic DNA (such as DNA with a codon usage that has been specifically adapted for expression in the intended host cell or host organism).

According to one aspect of the invention, the nucleic acid of the invention is in essentially isolated from, as defined herein.

The nucleic acid of the invention may also be in the form of, be present in and/or be part of a vector, such as for example a plasmid, cosmid or YAC, which again may be in essentially isolated form.

The nucleic acids of the invention can be prepared or obtained in a manner known per se, based on the information on the amino acid sequences for the polypeptides of the invention given herein, and/or can be isolated from a suitable natural source. To provide analogs, nucleotide sequences encoding naturally occurring V_{HH} domains can for example be subjected to site-directed mutagenesis, so at to provide a nucleic acid of the invention encoding said analog. Also, as will be clear to the skilled person, to prepare a nucleic acid of the invention, also several nucleotide

sequences, such as at least one nucleotide sequence encoding a Nanobody® and for example nucleic acids encoding one or more linkers can be linked together in a suitable manner.

Techniques for generating the nucleic acids of the invention will be clear to the skilled person and may for instance include, but are not limited to, automated DNA synthesis; site-directed mutagenesis; combining two or more naturally occurring and/or synthetic sequences (or two or more parts thereof), introduction of mutations that lead to the expression of a truncated expression product; introduction of one or more restriction sites (e.g. to create cassettes and/or regions that may easily be digested and/or ligated using suitable restriction enzymes), and/or the introduction of mutations by means of a PCR reaction using one or more "mismatched" primers, using for example a sequence of a naturally occurring form of Dkk-1 as a template. These and other techniques will be clear to the skilled person, and reference is again made to the standard handbooks, such as Sambrook et al. and Ausubel et al., mentioned above, as well as the Examples below.

The nucleic acid of the invention may also be in the form of, be present in and/or be part of a genetic construct, as will be clear to the person skilled in the art and as described on pages 131-134 of WO 08/020079 (incorporated herein by reference). Such genetic constructs generally comprise at least one nucleic acid of the invention that is optionally linked to one or more elements of genetic constructs known per se, such as for example one or more suitable regulatory elements (such as a suitable promoter(s), enhancer(s), terminator(s), etc.) and the further elements of genetic constructs referred to herein. Such genetic constructs comprising at least one nucleic acid of the invention will also be referred to herein as "genetic constructs of the invention".

The genetic constructs of the invention may be DNA or RNA, and are preferably double-stranded DNA. The genetic constructs of the invention may also be in a form suitable for transformation of the intended host cell or host organism, in a form suitable for integration into the genomic DNA of the intended host cell or in a form suitable for independent replication, maintenance and/or inheritance in the intended host organism. For instance, the genetic constructs of the invention may be in the form of a vector, such as for example a plasmid, cosmid, YAC, a viral vector or transposon. In particular, the vector may be an expression vector, i.e. a vector that can provide for expression in vitro and/or in vivo (e.g. in a suitable host cell, host organism and/or expression system).

In a preferred but non-limiting aspect, a genetic construct of the invention comprises

i) at least one nucleic acid of the invention; operably connected to

ii) one or more regulatory elements, such as a promoter and optionally a suitable terminator;

and optionally also

iii) one or more further elements of genetic constructs known per se;

in which the terms "operably connected" and "operably linked" have the meaning given on pages 131-134 of WO 08/020079; and in which the "regulatory elements", "promoter", "terminator" and "further elements" are as described on pages 131-134 of WO 08/020079; and in which the genetic constructs may further be as described on pages 131-134 of WO 08/020079.

The nucleic acids of the invention and/or the genetic constructs of the invention may be used to transform a host cell or host organism, i.e. for expression and/or production of the amino acid sequence, Nanobody® or polypeptide of the invention. Suitable hosts or host cells will be clear to the skilled person, and may for example be any suitable fungal, prokaryotic or eukaryotic cell or cell line or any suitable fungal, prokaryotic or eukaryotic organism, for example those described on pages 134 and 135 of WO 08/020079.; as well as all other hosts or host cells known per se for the expression and production of antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments), which will be clear to the skilled person. Reference is also made to the general background art cited hereinabove, as well as to for example WO 94/29457; WO 96/34103; WO 99/42077; Frenken et al., (1998), supra; Riechmann and Muyldermans, (1999), supra; van der Linden, (2000), supra; Thomassen et al., (2002), supra; Joosten et al., (2003), supra; Joosten et al., (2005), supra; and the further references cited herein.

The amino acid sequences, Nanobodies® and polypeptides of the invention can also be introduced and expressed in one or more cells, tissues or organs of a multicellular organism, for example for prophylactic and/or therapeutic purposes (e.g. as a gene therapy), as further described on pages 135 and 136 of in WO 08/020079and in the further references cited in WO 08/020079.

For expression of the Nanobodies® in a cell, they may also be expressed as so-called "intrabodies", as for example described in WO 94/02610, WO 95/22618 and US-A-7004940; WO 03/014960; in Cattaneo, A. & Biocca, S. (1997) Intracellular Antibodies: Development and Applications. Landes and Springer-Verlag; and in Kontermann, Methods 34, (2004), 163-170.

The amino acid sequences, Nanobodies® and polypeptides of the invention can for example also be produced in the milk of transgenic mammals, for example in the milk of rabbits, cows, goats or sheep (see for example US-A-6,741,957, US-A-6,304,489 and US-A-6,849,992 for general techniques for introducing transgenes into mammals), in plants or parts of plants including but not limited to their leaves, flowers, fruits, seed, roots or turbers (for example in tobacco, maize, soybean or alfalfa) or in for example pupae of the silkworm *Bombix mori*.

Furthermore, the amino acid sequences, Nanobodies® and polypeptides of the invention can also be expressed and/or produced in cell-free expression systems, and suitable examples of such systems will be clear to the skilled person. Some preferred, but non-limiting examples include expression in the wheat germ system; in rabbit reticulocyte lysates; or in the *E. coli* Zubay system.

As mentioned above, one of the advantages of the use of Nanobodies® is that the polypeptides based thereon can be prepared through expression in a suitable bacterial system, and suitable bacterial expression systems, vectors, host cells, regulatory elements, etc., will be clear to the skilled person, for example from the references cited above. It should however be noted that the invention in its broadest sense is not limited to expression in bacterial systems.

Preferably, in the invention, an (in vivo or in vitro) expression system, such as a bacterial expression system, is used that provides the polypeptides of the invention in a form that is suitable for pharmaceutical use, and such expression systems will again be clear to the skilled person. As also will be clear to the skilled person, polypeptides of the invention suitable for pharmaceutical use can be prepared using techniques for peptide synthesis.

For production on industrial scale, preferred heterologous hosts for the (industrial) production of Nanobodies® or Nanobody®-containing protein therapeutics include strains of *E. coli*, *Pichia pastoris*, *S. cerevisiae* that are suitable for large scale expression/production/fermentation, and in particular for large scale pharmaceutical (i.e. GMP grade) expression/production/fermentation. Suitable examples of such strains will be clear to the skilled person. Such strains and production/expression systems are also made available by companies such as Biovitrum (Uppsala, Sweden).

Alternatively, mammalian cell lines, in particular Chinese hamster ovary (CHO) cells, can be used for large scale expression/production/fermentation, and in particular for large scale pharmaceutical expression/production/fermentation. Again, such expression/production systems are also made available by some of the companies mentioned above.

The choice of the specific expression system would depend in part on the requirement for certain post-translational modifications, more specifically glycosylation. The production of a Nanobody®-containing recombinant protein for which glycosylation is desired or required would necessitate the use of mammalian expression hosts that have the ability to glycosylate the expressed protein. In this respect, it will be clear to the skilled person that the glycosylation pattern obtained (i.e. the kind, number and position of residues attached) will depend on the cell or cell line that is used for the expression. Preferably, either a human cell or cell line is used (i.e. leading to a protein that essentially has a human glycosylation pattern) or another mammalian cell line is used that can provide a glycosylation pattern that is essentially and/or functionally the same as human glycosylation or at least mimics human glycosylation. Generally, prokaryotic hosts such as *E. coli* do not have the ability to glycosylate proteins, and the use of lower eukaryotes such as yeast usually leads to a glycosylation pattern that differs from human glycosylation. Nevertheless, it should be understood that all the foregoing host cells and expression systems can be used in the invention, depending on the desired amino acid sequence, Nanobody® or polypeptide to be obtained.

Thus, according to one non-limiting aspect of the invention, the amino acid sequence, Nanobody® or polypeptide of the invention is glycosylated. According to another non-limiting aspect of the invention, the amino acid sequence, Nanobody® or polypeptide of the invention is non-glycosylated.

According to one preferred, but non-limiting aspect of the invention, the amino acid sequence, Nanobody® or polypeptide of the invention is produced in a bacterial cell, in particular a bacterial cell suitable for large scale pharmaceutical production, such as cells of the strains mentioned above.

According to another preferred, but non-limiting aspect of the invention, the amino acid sequence, Nanobody® or polypeptide of the invention is produced in a yeast cell, in particular a yeast cell suitable for large scale pharmaceutical production, such as cells of the species mentioned above.

According to yet another preferred, but non-limiting aspect of the invention, the amino acid sequence, Nanobody® or polypeptide of the invention is produced in a mammalian cell, in particular in a human cell or in a cell of a human cell line, and more in particular in a human cell or in a cell of a human cell line that is suitable for large scale pharmaceutical production, such as the cell lines mentioned hereinabove.

As further described on pages 138 and 139 of WO 08/020079, when expression in a host cell is used to produce the amino acid sequences, Nanobodies® and the polypeptides of the invention, the amino acid sequences, Nanobodies® and polypeptides of the invention can be produced either intracellullarly (e.g. in the cytosol, in the periplasma or in inclusion bodies) and then isolated from the host cells and optionally further purified; or can be produced extracellularly (e.g. in the medium in which the host cells are cultured) and then isolated from the culture medium and optionally further purified. Thus, according to one non-limiting aspect of the invention, the amino acid sequence, Nanobody® or polypeptide of the invention is an amino acid sequence, Nanobody® or polypeptide that has been produced intracellularly and that has been isolated from the host cell, and in particular from a bacterial cell or from an inclusion body in a bacterial cell. According to another non-limiting aspect of the invention, the amino acid sequence, Nanobody® or polypeptide of the invention is an amino acid sequence, Nanobody® or polypeptide that has been produced extracellularly, and that has been isolated from the medium in which the host cell is cultivated.

Some preferred, but non-limiting promoters for use with these host cells include those mentioned on pages 139 and 140 of WO 08/020079.

Some preferred, but non-limiting secretory sequences for use with these host cells include those mentioned on page 140 of WO 08/020079.

Suitable techniques for transforming a host or host cell of the invention will be clear to the skilled person and may depend on the intended host cell/host organism and the genetic construct to be used. Reference is again made to the handbooks and patent applications mentioned above.

After transformation, a step for detecting and selecting those host cells or host organisms that have been succesfully transformed with the nucleotide sequence/genetic construct of the invention may be performed. This may for instance be a selection step based on a selectable marker present in the genetic construct of the invention or a step involving the detection of the amino acid sequence of the invention, e.g. using specific antibodies.

The transformed host cell (which may be in the form or a stable cell line) or host organisms (which may be in the form of a stable mutant line or strain) form further aspects of the present invention.

Preferably, these host cells or host organisms are such that they express, or are (at least) capable of expressing (e.g. under suitable conditions), an amino acid sequence, Nanobody® or polypeptide of the invention (and in case of a host organism: in at least one cell, part, tissue or organ thereof). The invention also includes further generations, progeny and/or offspring of the host cell or host organism of the invention, that may for instance be obtained by cell division or by sexual or asexual reproduction.

To produce/obtain expression of the amino acid sequences of the invention, the transformed host cell or transformed host organism may generally be kept, maintained and/or cultured under conditions such that the (desired) amino acid sequence, Nanobody® or polypeptide of the invention is expressed/produced. Suitable conditions will be clear to the skilled person and will usually depend upon the host cell/host organism used, as well as on the regulatory elements that control the expression of the (relevant) nucleotide sequence of the invention. Again, reference is made to the handbooks and patent applications mentioned above in the paragraphs on the genetic constructs of the invention.

Generally, suitable conditions may include the use of a suitable medium, the presence of a suitable source of food and/or suitable nutrients, the use of a suitable temperature, and optionally the presence of a suitable inducing factor or compound (e.g. when the nucleotide sequences of the invention are under the control of an inducible promoter); all of which may be selected by the skilled person. Again, under such conditions, the amino acid sequences of the invention may be expressed in a constitutive manner, in a transient manner, or only when suitably induced.

It will also be clear to the skilled person that the amino acid sequence, Nanobody® or polypeptide of the invention may (first) be generated in an immature form (as mentioned above), which may then be subjected to post-translational modification, depending on the host cell/host

organism used. Also, the amino acid sequence, Nanobody® or polypeptide of the invention may be glycosylated, again depending on the host cell/host organism used.

The amino acid sequence, Nanobody® or polypeptide of the invention may then be isolated from the host cell/host organism and/or from the medium in which said host cell or host organism was cultivated, using protein isolation and/or purification techniques known per se, such as (preparative) chromatography and/or electrophoresis techniques, differential precipitation techniques, affinity techniques (e.g. using a specific, cleavable amino acid sequence fused with the amino acid sequence, Nanobody® or polypeptide of the invention) and/or preparative immunological techniques (i.e. using antibodies against the amino acid sequence to be isolated).

Generally, for pharmaceutical use, the polypeptides of the invention may be formulated as a pharmaceutical preparation or compositions comprising at least one polypeptide of the invention and at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally one or more further pharmaceutically active polypeptides and/or compounds. By means of non-limiting examples, such a formulation may be in a form suitable for oral administration, for parenteral administration (such as by intravenous, intramuscular or subcutaneous injection or intravenous infusion), for topical administration, for administration by inhalation, by a skin patch, by an implant, by a suppository, etc.. Such suitable administration forms - which may be solid, semi-solid or liquid, depending on the manner of administration - as well as methods and carriers for use in the preparation thereof, will be clear to the skilled person, and are further described herein.

Thus, in a further aspect, the invention relates to a pharmaceutical composition that contains at least one amino acid of the invention, at least one Nanobody® of the invention or at least one polypeptide of the invention and at least one suitable carrier, diluent or excipient (i.e. suitable for pharmaceutical use), and optionally one or more further active substances.

Generally, the amino acid sequences, Nanobodies® and polypeptides of the invention can be formulated and administered in any suitable manner known per se, for which reference is for example made to the general background art cited above (and in particular to WO 04/041862, WO 04/041863, WO 04/041865, WO 04/041867 and WO 08/020079) as well as to the standard handbooks, such as Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Company, USA (1990), Remington, the Science and Practice of Pharmacy, 21th Edition, Lippincott Williams and Wilkins (2005); or the Handbook of Therapeutic Antibodies (S. Dubel, Ed.), Wiley, Weinheim, 2007 (see for example pages 252-255).

For example, the amino acid sequences, Nanobodies® and polypeptides of the invention may be formulated and administered in any manner known per se for conventional antibodies and antibody fragments (including ScFv's and diabodies) and other pharmaceutically active proteins. Such formulations and methods for preparing the same will be clear to the skilled person, and for example

include preparations suitable for parenteral administration (for example intravenous, intraperitoneal, subcutaneous, intramuscular, intraluminal, intra-arterial or intrathecal administration) or for topical (i.e. transdermal or intradermal) administration.

Preparations for parenteral administration may for example be sterile solutions, suspensions, dispersions or emulsions that are suitable for infusion or injection. Suitable carriers or diluents for such preparations for example include, without limitation, those mentioned on page 143 of WO 08/020079. Usually, aqueous solutions or suspensions will be preferred.

The amino acid sequences, Nanobodies® and polypeptides of the invention can also be administered using gene therapy methods of delivery. See, *e.g.*, U.S. Patent No. 5,399,346, which is incorporated by reference in its entirety. Using a gene therapy method of delivery, primary cells transfected with the gene encoding an amino acid sequence, Nanobody® or polypeptide of the invention can additionally be transfected with tissue specific promoters to target specific organs, tissue, grafts, tumors, or cells and can additionally be transfected with signal and stabilization sequences for subcellularly localized expression.

Thus, the amino acid sequences, Nanobodies® and polypeptides of the invention may be systemically administered, *e.g.*, orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the amino acid sequences, Nanobodies® and polypeptides of the invention may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of the amino acid sequence, Nanobody® or polypeptide of the invention. Their percentage in the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of the amino acid sequence, Nanobody® or polypeptide of the invention in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain binders, excipients, disintegrating agents, lubricants and sweetening or flavouring agents, for example those mentioned on pages 143-144 of WO 08/020079. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the amino acid sequences, Nanobodies® and polypeptides of the invention, sucrose or fructose as a sweetening agent, methyl and

propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the amino acid sequences, Nanobodies® and polypeptides of the invention may be incorporated into sustained-release preparations and devices.

Preparations and formulations for oral administration may also be provided with an enteric coating that will allow the constructs of the invention to resist the gastric environment and pass into the intestines. More generally, preparations and formulations for oral administration may be suitably formulated for delivery into any desired part of the gastrointestinal tract. In addition, suitable suppositories may be used for delivery into the gastrointestinal tract.

The amino acid sequences, Nanobodies® and polypeptides of the invention may also be administered intravenously or intraperitoneally by infusion or injection, as further described on pages 144 and 145 of WO 08/020079.

For topical administration, the amino acid sequences, Nanobodies® and polypeptides of the invention may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid, as further described on page 145 of WO 08/020079.

Generally, the concentration of the amino acid sequences, Nanobodies® and polypeptides of the invention in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the amino acid sequences, Nanobodies® and polypeptides of the invention required for use in treatment will vary not only with the particular amino acid sequence, Nanobody® or polypeptide selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician. Also the dosage of the amino acid sequences, Nanobodies® and polypeptides of the invention varies depending on the target cell, tumor, tissue, graft, or organ.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, *e.g.*, into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

An administration regimen could include long-term, daily treatment. By "long-term" is meant at least two weeks and preferably, several weeks, months, or years of duration. Necessary

modifications in this dosage range may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. See Remington's Pharmaceutical Sciences (Martin, E.W., ed. 4), Mack Publishing Co., Easton, PA. The dosage can also be adjusted by the individual physician in the event of any complication.

In another aspect, the invention relates to a method for the prevention and/or treatment of at least one Dkk-1-associated diseases or disorders, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence of the invention, of a Nanobody® of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

In the context of the present invention, the term "prevention and/or treatment" not only comprises preventing and/or treating the disease, but also generally comprises preventing the onset of the disease, slowing or reversing the progress of disease, preventing or slowing the onset of one or more symptoms associated with the disease, reducing and/or alleviating one or more symptoms associated with the disease, reducing the severity and/or the duration of the disease and/or of any symptoms associated therewith and/or preventing a further increase in the severity of the disease and/or of any symptoms associated therewith, preventing, reducing or reversing any physiological damage caused by the disease, and generally any pharmacological action that is beneficial to the patient being treated.

The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases and disorders mentioned herein.

The invention relates to a method for the prevention and/or treatment of at least one disease or disorder that is associated with Dkk-1, with its biological or pharmacological activity, and/or with the biological pathways or (the inhibiting effect on) signaling in which Dkk-1 is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence of the invention, of a Nanobody® of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same. In particular, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder that can be treated by modulating Dkk-1, its biological or pharmacological activity, and/or the biological pathways or (the inhibiting effect on) signaling in which Dkk-1 is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence of the invention, of a Nanobody® of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same. In particular, said pharmaceutically effective amount may be an amount that is sufficient to modulate

Dkk-1, its biological or pharmacological activity, and/or the biological pathways or (the inhibiting effect on) signaling in which Dkk-1 is involved; and/or an amount that provides a level of the amino acid sequence of the invention, of a Nanobody® of the invention, of a polypeptide of the invention in the circulation that is sufficient to modulate Dkk-1, its biological or pharmacological activity, and/or the biological pathways or (the inhibiting effect on) signaling in which Dkk-1 is involved.

The invention furthermore relates to a method for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering an amino acid sequence of the invention, a Nanobody® of the invention or a polypeptide of the invention to a patient, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence of the invention, of a Nanobody® of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

More in particular, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder chosen from the group consisting of the diseases and disorders listed herein, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence of the invention, of a Nanobody® of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

In another aspect, the invention relates to a method for immunotherapy, and in particular for passive immunotherapy, which method comprises administering, to a subject suffering from or at risk of the diseases and disorders mentioned herein, a pharmaceutically active amount of an amino acid sequence of the invention, of a Nanobody® of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

In the above methods, the amino acid sequences, Nanobodies® and/or polypeptides of the invention and/or the compositions comprising the same can be administered in any suitable manner, depending on the specific pharmaceutical formulation or composition to be used. Thus, the amino acid sequences, Nanobodies® and/or polypeptides of the invention and/or the compositions comprising the same can for example be administered orally, intraperitoneally (e.g. intravenously, subcutaneously, intramuscularly, or via any other route of administration that circumvents the gastrointestinal tract), intranasally, transdermally, topically, by means of a suppository, by inhalation, again depending on the specific pharmaceutical formulation or composition to be used. The clinician will be able to select a suitable route of administration and a suitable pharmaceutical formulation or composition to be used in such administration, depending on the disease or disorder to be prevented or treated and other factors well known to the clinician.

The amino acid sequences, Nanobodies® and/or polypeptides of the invention and/or the compositions comprising the same are administered according to a regime of treatment that is suitable for preventing and/or treating the disease or disorder to be prevented or treated. The

clinician will generally be able to determine a suitable treatment regimen, depending on factors such as the disease or disorder to be prevented or treated, the severity of the disease to be treated and/or the severity of the symptoms thereof, the specific amino acid sequence, Nanobody® or polypeptide of the invention to be used, the specific route of administration and pharmaceutical formulation or composition to be used, the age, gender, weight, diet, general condition of the patient, and similar factors well known to the clinician.

Generally, the treatment regimen will comprise the administration of one or more amino acid sequences, Nanobodies® and/or polypeptides of the invention, or of one or more compositions comprising the same, in one or more pharmaceutically effective amounts or doses. The specific amount(s) or doses to administered can be determined by the clinician, again based on the factors cited above.

Generally, for the prevention and/or treatment of the diseases and disorders mentioned herein and depending on the specific disease or disorder to be treated, the potency of the specific amino acid sequence, Nanobody® and polypeptide of the invention to be used, the specific route of administration and the specific pharmaceutical formulation or composition used, the amino acid sequences, Nanobodies® and polypeptides of the invention will generally be administered in an amount between 1 gram and 0.01 microgram per kg body weight per day, preferably between 0.1 gram and 0.1 microgram per kg body weight per day, such as about 1, 10, 100 or 1000 microgram per kg body weight per day, either continuously (e.g. by infusion), as a single daily dose or as multiple divided doses during the day. The clinician will generally be able to determine a suitable daily dose, depending on the factors mentioned herein. It will also be clear that in specific cases, the clinician may choose to deviate from these amounts, for example on the basis of the factors cited above and his expert judgment. Generally, some guidance on the amounts to be administered can be obtained from the amounts usually administered for comparable conventional antibodies or antibody fragments against the same target administered via essentially the same route, taking into account however differences in affinity/avidity, efficacy, biodistribution, half-life and similar factors well known to the skilled person.

Usually, in the above method, a single amino acid sequence, Nanobody® or polypeptide of the invention will be used. It is however within the scope of the invention to use two or more amino acid sequences, Nanobodies® and/or polypeptides of the invention in combination.

The Nanobodies®, amino acid sequences and polypeptides of the invention may also be used in combination with one or more further pharmaceutically active compounds or principles, i.e. as a combined treatment regimen, which may or may not lead to a synergistic effect. Again, the clinician will be able to select such further compounds or principles, as well as a suitable combined treatment regimen, based on the factors cited above and his expert judgement.

In particular, the amino acid sequences, Nanobodies® and polypeptides of the invention may be used in combination with other pharmaceutically active compounds or principles that are or can be used for the prevention and/or treatment of the diseases and disorders cited herein, as a result of which a synergistic effect may or may not be obtained. Examples of such compounds and principles, as well as routes, methods and pharmaceutical formulations or compositions for administering them will be clear to the clinician.

When two or more substances or principles are to be used as part of a combined treatment regimen, they can be administered via the same route of administration or via different routes of administration, at essentially the same time or at different times (e.g. essentially simultaneously, consecutively, or according to an alternating regime). When the substances or principles are to be administered simultaneously via the same route of administration, they may be administered as different pharmaceutical formulations or compositions or part of a combined pharmaceutical formulation or composition, as will be clear to the skilled person.

Also, when two or more active substances or principles are to be used as part of a combined treatment regimen, each of the substances or principles may be administered in the same amount and according to the same regimen as used when the compound or principle is used on its own, and such combined use may or may not lead to a synergistic effect. However, when the combined use of the two or more active substances or principles leads to a synergistic effect, it may also be possible to reduce the amount of one, more or all of the substances or principles to be administered, while still achieving the desired therapeutic action. This may for example be useful for avoiding, limiting or reducing any unwanted side-effects that are associated with the use of one or more of the substances or principles when they are used in their usual amounts, while still obtaining the desired pharmaceutical or therapeutic effect.

The effectiveness of the treatment regimen used according to the invention may be determined and/or followed in any manner known per se for the disease or disorder involved, as will be clear to the clinician. The clinician will also be able, where appropriate and on a case-by-case basis, to change or modify a particular treatment regimen, so as to achieve the desired therapeutic effect, to avoid, limit or reduce unwanted side-effects, and/or to achieve an appropriate balance between achieving the desired therapeutic effect on the one hand and avoiding, limiting or reducing undesired side effects on the other hand.

Generally, the treatment regimen will be followed until the desired therapeutic effect is achieved and/or for as long as the desired therapeutic effect is to be maintained. Again, this can be determined by the clinician.

In another aspect, the invention relates to the use of an amino acid sequence, Nanobody® or polypeptide of the invention in the preparation of a pharmaceutical composition for prevention

and/or treatment of at least one Dkk-1-associated disease or disorder; and/or for use in one or more of the methods of treatment mentioned herein.

The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases and disorders mentioned herein.

The invention also relates to the use of an amino acid sequence, Nanobody® or polypeptide of the invention in the preparation of a pharmaceutical composition for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering an amino acid sequence, Nanobody® or polypeptide of the invention to a patient.

More in particular, the invention relates to the use of an amino acid sequence, Nanobody® or polypeptide of the invention in the preparation of a pharmaceutical composition for the prevention and/or treatment of at least one Dkk-1-associated disease or disorder, and in particular for the prevention and treatment of one or more of the diseases and disorders listed herein.

Again, in such a pharmaceutical composition, the one or more amino acid sequences, Nanobodies® or polypeptides of the invention may also be suitably combined with one or more other active principles, such as those mentioned herein.

Finally, although the use of the Nanobodies® of the invention (as defined herein) and of the polypeptides of the invention is much preferred, it will be clear that on the basis of the description herein, the skilled person will also be able to design and/or generate, in an analogous manner, other amino acid sequences and in particular (single) domain antibodies against Dkk-1, as well as polypeptides comprising such (single) domain antibodies.

For example, it will also be clear to the skilled person that it may be possible to "graft" one or more of the CDR's mentioned above for the Nanobodies® of the invention onto such (single) domain antibodies or other protein scaffolds, including but not limited to human scaffolds or non-immunoglobulin scaffolds. Suitable scaffolds and techniques for such CDR grafting will be clear to the skilled person and are well known in the art, see for example those mentioned in WO 08/020079. For example, techniques known per se for grafting mouse or rat CDR's onto human frameworks and scaffolds can be used in an analogous manner to provide chimeric proteins comprising one or more of the CDR's of the Nanobodies® of the invention and one or more human framework regions or sequences.

It should also be noted that, when the Nanobodies® of the inventions contain one or more other CDR sequences than the preferred CDR sequences mentioned above, these CDR sequences can be obtained in any manner known per se, for example using one or more of the techniques described in WO 08/020079.

Further uses of the amino acid sequences, Nanobodies®, polypeptides, nucleic acids, genetic constructs and hosts and host cells of the invention will be clear to the skilled person based on the disclosure herein. For example, and without limitation, the amino acid sequences of the invention can be linked to a suitable carrier or solid support so as to provide a medium than can be used in a manner known per se to purify Dkk-1 from compositions and preparations comprising the same. Derivatives of the amino acid sequences of the invention that comprise a suitable detectable label can also be used as markers to determine (qualitatively or quantitatively) the presence of Dkk-1 in a composition or preparation or as a marker to selectively detect the presence of Dkk-1 on the surface of a cell or tissue (for example, in combination with suitable cell sorting techniques).

The invention will now be further described by means of the following non-limiting preferred aspects, examples and figures:

The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced hereinabove.

Preferred Aspects:

Aspect A-1. An amino acid sequence that is directed against and/or that can specifically bind to Dkk-1.

Aspect A-2. An amino acid sequence according to aspect A-1, that can specifically bind Dkk-1 but not Dkk-2, Dkk-3 or Dkk-4.

Aspect A-3. An amino acid sequence according to aspect A-1, that can specifically bind Dkk-1, Dkk-2 and Dkk-4 but not Dkk-3; or that can specifically bind Dkk-1 and Dkk-4 but not Dkk-2 and Dkk-3.

Aspect A-4. An amino acid sequence according to any of aspects A-1 to A-3, that inhibits and/or blocks binding of LRP5/6 to Dkk-1.

Aspect A-5. An amino acid sequence according to any of aspects A-1 to A-4, that competes with LRP5/6 for binding to Dkk-1.

Aspect A-6. An amino acid sequence according to any of aspects A-1 to A-5, that binds to the LRP5/6 binding site on Dkk-1.

Aspect A-7. An amino acid sequence according to any of aspects A-1 to A-6, that binds to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 on Dkk-1.

Aspect A-8. An amino acid sequence according to any of aspects A-1 to A-7, that binds to at least one of amino acid residues R242 or H210 on Dkk-1.

Aspect A-9. An amino acid sequence according to any of aspects A-1 to A-8, that inhibits and/or blocks binding of BHQ880 to Dkk-1.

Aspect A-10. An amino acid sequence according to any of aspects A-1 to A-9, that competes with BHQ880 for binding to Dkk-1.

Aspect A-11. An amino acid sequence according to any of aspects A-1 to A-10, that binds to the BHQ880 binding site on Dkk-1.

Aspect A-12. An amino acid sequence according to any of aspects A-1 to A-11, that inhibits and/or blocks binding of RH2-18 to Dkk-1.

Aspect A-13. An amino acid sequence according to any of aspects A-1 to A-12, that competes with RH2-18 for binding to Dkk-1.

Aspect A-14. An amino acid sequence according to any of aspects A-1 to A-13, that binds to the RH2-18 binding site on Dkk-1.

Aspect A-15. An amino acid sequence according to any of aspects A-1 to A-14, that inhibits and/or blocks binding of 11H10 to Dkk-1.

Aspect A-16. An amino acid sequence according to any of aspects A-1 to A-15, that competes with 11H10 for binding to Dkk-1.

Aspect A-17. An amino acid sequence according to any of aspects A-1 to A-16, that binds to the 11H10 binding site on Dkk-1.

Aspect A-18. An amino acid sequence according to any of aspects A-1 to A-17, that inhibits and/or blocks binding of Kremen to Dkk-1.

Aspect A-19. An amino acid sequence according to any of aspects A-1 to A-18, that competes with Kremen for binding to Dkk-1.

Aspect A-20. An amino acid sequence according to any of aspects A-1 to A-19, that binds to the Kremen binding site on Dkk-1.

Aspect A-21. An amino acid sequence according to any of aspects A-1 to A-20, that binds to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1.

Aspect A-22. An amino acid sequence according to any of aspects A-1 to A-21, that binds to at least one of amino acid residues R197 or K232 on Dkk-1.

Aspect A-23. An amino acid sequence according to any of aspects A-1 to A-22, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway.

Aspect A-24. An amino acid sequence according to aspects A-23, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than BHQ880.

Aspect A-25. An amino acid sequence according to aspects A-23, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than RH2-18.

Aspect A-26. An amino acid sequence according to aspects A-23, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than 11H10.

Aspect A-27. An amino acid sequence according to any of aspects A-1 to A-26, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction.

Aspect A-28. An amino acid sequence according to aspects A-27, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than BHQ880.

Aspect A-29. An amino acid sequence according to aspects A-27, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than RH2-18.

Aspect A-30. An amino acid sequence according to aspects A-27, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than 11H10.

Aspect A-31. An amino acid sequence according to any of aspects A-1 to A-30, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation.

Aspect A-32. An amino acid sequence according to aspects A-31, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than BHQ880.

Aspect A-33. An amino acid sequence according to aspects A-31, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than RH2-18.

Aspect A-34. An amino acid sequence according to aspects A-31, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than 11H10.

Aspect A-35. An amino acid sequence according to any of aspects A-1 to A-34, that increases bone mass in a subject.

Aspect A-36. An amino acid sequence according to aspect A-35, that increases bone mass in a subject equally or better than BHQ880.

Aspect A-37. An amino acid sequence according to aspect A-35, that increases bone mass in a subject equally or better than RH2-18.

Aspect A-38. An amino acid sequence according to aspect A-35, that increases bone mass in a subject equally or better than 11H10.

Aspect A-39. An amino acid sequence according to any of aspects A-1 to A-38, that reduces bone lesions.

Aspect A-40. An amino acid sequence according to aspect A-39, that reduces bone lesions equally or better than BHQ880.

Aspect A-41. An amino acid sequence according to aspect A-39, that reduces bone lesions equally or better than RH2-18.

Aspect A-42. An amino acid sequence according to aspect A-39, that reduces bone lesions equally or better than 11H10.

Aspect A-43. An amino acid sequence according to any of aspects A-1 to A-42, that reduces tumor burden.

Aspect A-44. An amino acid sequence according to aspect A-43, that reduces tumor burden equally or better than BHQ880.

Aspect A-45. An amino acid sequence according to aspect A-43, that reduces tumor burden equally or better than RH2-18.

Aspect A-46. An amino acid sequence according to aspect A-43, that reduces tumor burden equally or better than 11H10.

Aspect A-47. An amino acid sequence according to any of aspects A-1 to A-46, that modulates the inhibitory effect of Dkk-1 on Wnt signaling.

Aspect A-48. An amino acid sequence according to aspect A-47, wherein said amino acid sequence modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as BHQ880.

Aspect A-49. An amino acid sequence according to aspect A-47, wherein said amino acid sequence modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as RH2-18.

Aspect A-50. An amino acid sequence according to aspect A-47, wherein said amino acid sequence modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as 11H10.

Aspect A-51. An amino acid sequence according to any of aspects A-1 to A-50, that is in essentially isolated form.

Aspect A-52. An amino acid sequence according to aspect A-1 or A-51, for administration to a subject, wherein said amino acid sequence does not naturally occur in said subject.

Aspect A-53. An amino acid sequence that can specifically bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/litre or less, and preferably 10^{-7} to 10^{-12} moles/litre or less and more preferably 10^{-8} to 10^{-12} moles/litre. Such an amino acid sequence may in particular be an amino acid sequence according to any of the preceding aspects.

Aspect A-54. An amino acid sequence that can specifically bind to Dkk-1 with a rate of association (k_{on}-rate) of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$. Such an amino acid sequence may in particular be an amino acid sequence according to any of the preceding aspects.

Aspect A-55. An amino acid sequence that can specifically bind to Dkk-1 with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1}, preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}. Such an amino acid sequence may in particular be an amino acid sequence according to any of the preceding aspects.

Aspect A-56. An amino acid sequence that can specifically bind to Dkk-1 with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM. Such an amino acid sequence may in particular be an amino acid sequence according to any of the preceding aspects.

Aspect A-57. An amino acid sequence according to any of the preceding aspects, that is a naturally occurring amino acid sequence (from any suitable species) or a synthetic or semi-synthetic amino acid sequence.

Aspect A-58. An amino acid sequence according to any of the preceding aspects, that comprises an immunoglobulin fold or that under suitable conditions is capable of forming an immunoglobulin fold.

Aspect A-59. An amino acid sequence according to any of the preceding aspects, that essentially consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively).

Aspect A-60. An amino acid sequence according to any of the preceding aspects, that is an immunoglobulin sequence.

Aspect A-61. An amino acid sequence according to any of the preceding aspects, that is a naturally occurring immunoglobulin sequence (from any suitable species) or a synthetic or semi-synthetic immunoglobulin sequence.

Aspect A-62. An amino acid sequence according to any of the preceding aspects that is a humanized immunoglobulin sequence, a camelized immunoglobulin sequence or an immunoglobulin sequence that has been obtained by techniques such as affinity maturation.

Aspect A-63. An amino acid sequence according to any of the preceding aspects, that essentially consists of a light chain variable domain sequence (e.g. a VL-sequence); or of a heavy chain variable domain sequence (e.g. a VH-sequence).

Aspect A-64. An amino acid sequence according to any of the preceding aspects, that essentially consists of a heavy chain variable domain sequence that is derived from a conventional four-chain antibody or that essentially consist of a heavy chain variable domain sequence that is derived from heavy chain antibody.

Aspect A-65. An amino acid sequence according to any of the preceding aspects, that essentially consists of a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody), of a single domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody), of a "dAb" (or an amino acid sequence that is suitable for use as a dAb) or of a Nanobody® (including but not limited to a VHH sequence).

Aspect A-66. An amino acid sequence according to any of the preceding aspects, that essentially consists of a Nanobody®.

Aspect A-67. An amino acid sequence according to any of the preceding aspects, that essentially consists of a Nanobody® that

i) has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1 to 22, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

Aspect A-68. An amino acid sequence according to any of the preceding aspects, that essentially consists of a polypeptide that

i) has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1817-1820], in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

Aspect A-69. An amino acid sequence according to any of the preceding aspects, that essentially consists of a Nanobody® that

i) has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

Aspect A-70. An amino acid sequence according to any of the preceding aspects, that essentially consists of a humanized Nanobody®.

Aspect A-71. An amino acid sequence according to any of the preceding aspects, that in addition to the at least one binding site for binding against Dkk-1, contains one or more further binding sites for binding against other antigens, proteins or targets.

Aspect B-1. An amino acid sequence that is directed against and/or that can specifically bind Dkk-1, and that comprises one or more stretches of amino acid residues chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 335-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

or any suitable combination thereof.

Such an amino acid sequence may in particular be an amino acid sequence according to any of the aspects A-1 to A-71.

Aspect B-2. An amino acid sequence according to aspect B-1, in which at least one of said stretches of amino acid residues forms part of the antigen binding site for binding against Dkk-1.

Aspect B-3. An amino acid sequence that is directed against and/or that can specifically bind Dkk-1 and that comprises two or more stretches of amino acid residues chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 335-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

such that (i) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to a), b) or c), the second stretch of amino acid residues corresponds to one of the amino acid sequences according to d), e), f), g), h) or i); (ii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to d), e) or f), the second stretch of amino acid residues corresponds to one of the amino acid sequences according to a), b), c), g), h) or i); or (iii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to g), h) or i), the second stretch of amino acid residues corresponds to one of the amino acid sequences according to a), b), c), d), e) or f).

Such an amino acid sequence may in particular be an amino acid sequence according to any of the aspects A-1 to A-71, B-1 or B-2.

Aspect B-4. An amino acid sequence according to aspect B-3, in which the at least two stretches of amino acid residues forms part of the antigen binding site for binding against Dkk-1.

Aspect B-5. An amino acid sequence that is directed against and/or that can specifically bind Dkk-1 and that comprises three or more stretches of amino acid residues, in which the first stretch of amino acid residues is chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 335-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

the second stretch of amino acid residues is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

and the third stretch of amino acid residues is chosen from the group consisting of:

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379.

Such an amino acid sequence may in particular be an amino acid sequence according to any of the aspects A-1 to A-71 and/or B-1 to B-4.

Aspect B-6. An amino acid sequence according to aspect B-5, in which the at least three stretches of amino acid residues forms part of the antigen binding site for binding against Dkk-1.

Aspect B-7. An amino acid sequence that is directed against and/or that can specifically bind Dkk-1 in which the CDR sequences of said amino acid sequence have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797. Such an amino acid sequence may in particular be an amino acid sequence according to any of the aspects A-1 to A-71 and/or B-1 to B-6.

Aspect C-1: An amino acid sequence that is directed against Dkk-1 and that cross-blocks the binding of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 to Dkk-1. Such an amino acid sequence may in particular be an amino acid sequence according to any of the aspects A-1 to A-71 and/or according to aspects B-1 to B-7. Also, preferably, such an amino acid sequence is able to specifically bind to Dkk-1.

Aspect C-2: An amino acid sequence that is directed against Dkk-1 and that is cross-blocked from binding to Dkk-1 by at least one of the amino acid sequences of SEQ ID NO's: 1589-1797. Such an amino acid sequence may in particular be an amino acid sequence according to any of the aspects A-1 to A-71 and/or according to aspects B-1 to B-7. Also, preferably, such an amino acid sequence is able to specifically bind to Dkk-1.

Aspect C-3: An amino acid sequence according to any of aspects C-1 or C-2, wherein the ability of said amino acid sequence to cross-block or to be cross-blocked is detected in a Biacore assay.

Aspect C-4: An amino acid sequence according to any of aspects C-1 to C-3 wherein the ability of said amino acid sequence to cross-block or to be cross-blocked is detected in an ELISA assay.

Aspect D-1: An amino acid sequence according to any of aspects B-1 to B-7 or C-1 to C-4, that is in essentially isolated form.

Aspect D-2: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, and/or D-1 for administration to a subject, wherein said amino acid sequence does not naturally occur in said subject.

Aspect D-3: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, and/or D-1 to D-2 that can specifically bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/litre or less, and preferably 10^{-7} to 10^{-12} moles/litre or less and more preferably 10^{-8} to 10^{-12} moles/litre.

Aspect D-4: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, and/or D-1 to D-3 that can specifically bind to Dkk-1 with a rate of association (k_{on}-rate) of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$.

Aspect D-5: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, and/or D-1 to D-4 that can specifically bind to Dkk-1 with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Aspect D-6: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, and/or D-1 to D-5 that can specifically bind to Dkk-1 with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

The amino acid sequences according to aspects D-1 to D-6 may in particular be an amino acid sequence according to any of the aspects A-1 to A-71.

Aspect E-1: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4 and/or D-1 to D-6, that is a naturally occurring amino acid sequence (from any suitable species) or a synthetic or semi-synthetic amino acid sequence.

Aspect E-2: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 that comprises an immunoglobulin fold or that under suitable conditions is capable of forming an immunoglobulin fold.

Aspect E-3: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or D-1 or D-2, that is an immunoglobulin sequence.

Aspect E-4: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-3, that is a naturally occurring immunoglobulin sequence (from any suitable species) or a synthetic or semi-synthetic immunoglobulin sequence.

Aspect E-5: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-4 that is a humanized immunoglobulin sequence, a camelized immunoglobulin sequence or an immunoglobulin sequence that has been obtained by techniques such as affinity maturation.

Aspect E-6: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-5 that essentially consists of a light chain variable domain sequence (e.g. a V_L-sequence); or of a heavy chain variable domain sequence (e.g. a V_H-sequence).

Aspect E-7: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-6, that essentially consists of a heavy chain variable domain sequence that is derived from a conventional four-chain antibody or that essentially consist of a heavy chain variable domain sequence that is derived from heavy chain antibody.

Aspect E-8: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-7, that essentially consists of a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody), of a single domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody), of a "dAb" (or an amino acid sequence that is suitable for use as a dAb) or of a Nanobody® (including but not limited to a V_{HH} sequence).

Aspect E-9: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-8 that essentially consists of a Nanobody®.

Aspect E-10: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-9 that essentially consists of a Nanobody® that

i) has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1 to 22, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

Aspect E-11: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-10, that essentially consists of a Nanobody® that

i) has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

Aspect E-12: An amino acid sequence according to any of aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-11that essentially consists of a humanized Nanobody®.

Aspect E-13: An amino acid sequence according to any of the aspects B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-12, that in addition to the at least one binding site for binding formed by the CDR sequences, contains one or more further binding sites for binding against other antigens, proteins or targets.

The amino acid sequences according to aspects E-1 to E-13 may in particular be an amino acid sequence according to any of the aspects A-1 to A-71.

Aspect F-1: An amino acid sequence that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

- CDR1 is chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 335-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

and/or

- CDR2 is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

and/or

- CDR3 is chosen from the group consisting of:

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379.

Such an amino acid sequence is preferably directed against Dkk-1 and/or an amino acid sequence that can specifically bind to Dkk-1. Also, such an amino acid sequence is preferably an amino acid sequence according to any of the aspects A-1 to A-71, C-1 to C-4, D-1 to D-6 and/or E-1 to E-13.

Aspect F-2: An amino acid sequence that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

- CDR1 is chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 335-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

and

- CDR2 is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

and

- CDR3 is chosen from the group consisting of:

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379.

Such an amino acid sequence is preferably directed against Dkk-1 and/or an amino acid sequence that can specifically bind to Dkk-1. Also, such an amino acid sequence is preferably an amino acid sequence according to any of the aspects A-1 to A-71, C-1 to C-4, D-1 to D-6 and/or E-1 to E-13.

Aspect F-3: An amino acid sequence according to any of aspects F-1 and F-2, in which the CDR sequences of said amino acid sequence have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797.

Such an amino acid sequence is preferably directed against Dkk-1 and/or an amino acid sequence that can specifically bind to Dkk-1. Also, such an amino acid sequence is preferably an amino acid sequence according to any of the aspects A-1 to A-71, C-1 to C-4, D-1 to D-6 and/or E-1 to E-13.

Aspect F-4: An amino acid sequence according to any of aspects F-1 to F-3 that is directed against Dkk-1 and that cross-blocks the binding of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797.

Aspect F-5: An amino acid sequence according to any of aspects F-1 to F-3 that is directed against Dkk-1 and that is cross-blocked from binding to Dkk-1 by at least one of the amino acid sequences of SEQ ID NO's: 1589-1797.

Aspect F-6: Amino acid sequence according to any of aspects F-4 or F-5 wherein the ability of said amino acid sequence to cross-block or to be cross-blocked is detected in a Biacore assay.

Aspect F-7: Amino acid sequence according to any of aspects F4 or F-5 wherein the ability of said amino acid sequence to cross-block or to be cross-blocked is detected in an ELISA assay.

Aspect F-8: An amino acid sequence according to any of aspects F-1 to F-7, that is in essentially isolated form.

Aspect F-9: An amino acid sequence according to any of aspects F-1 to F-8, for administration to a subject, wherein said an amino acid sequence does not naturally occur in said subject.

Aspect F-10: An amino acid sequence according to any of aspects F-1 to F-9, that can specifically bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/litre or less, and preferably 10^{-7} to 10^{-12} moles/litre or less and more preferably 10^{-8} to 10^{-12} moles/litre.

Aspect F-11: An amino acid sequence according to any of aspects F-1 to F-10, that can specifically bind to Dkk-1 with a rate of association (k_{on}-rate) of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$.

Aspect F-12: An amino acid sequence according to any of aspects F-1 to F-11, that can specifically bind to Dkk-1 with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Aspect F-13: An amino acid sequence according to any of aspects F-1 to F-12, that can specifically bind to Dkk-1 with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

Aspect F-14: An amino acid sequence according to any of aspects F-1 to F-13, that is a naturally occurring amino acid sequence (from any suitable species) or a synthetic or semi-synthetic amino acid sequence.

Aspect F-15: An amino acid sequence according to any of aspects F-1 to F-14, that comprises an immunoglobulin fold or that under suitable conditions is capable of forming an immunoglobulin fold.

Aspect F-16: An amino acid sequence according to any of aspects F-1 to F-15, that is an immunoglobulin sequence.

Aspect F-17: An amino acid sequence according to any of aspects F-1 to F-16, that is a naturally occurring immunoglobulin sequence (from any suitable species) or a synthetic or semi-synthetic immunoglobulin sequence.

Aspect F-18: An amino acid sequence according to any of aspects F-1 to F-17, that is a humanized immunoglobulin sequence, a camelized immunoglobulin sequence or an immunoglobulin sequence that has been obtained by techniques such as affinity maturation.

Aspect F-19: An amino acid sequence according to any of aspects F-1 to F-19, that essentially consists of a light chain variable domain sequence (e.g. a V_L-sequence); or of a heavy chain variable domain sequence (e.g. a V_H-sequence).

Aspect F-20: An amino acid sequence according to any of aspects F-1 to F-19, that essentially consists of a heavy chain variable domain sequence that is derived from a conventional four-chain antibody or that essentially consist of a heavy chain variable domain sequence that is derived from heavy chain antibody.

Aspect F-21: An amino acid sequence according to any of aspects F-1 to F-20, that essentially consists of a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody), of a single domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody), of a "dAb" (or an amino acid sequence that is suitable for use as a dAb) or of a Nanobody® (including but not limited to a V_{HH} sequence).

Aspect F-22: An amino acid sequence according to any of aspects F-1 to F-21, that essentially consists of a Nanobody®.

Aspect F-23: An amino acid sequence according to any of aspects F-1 to F-22, that essentially consists of a Nanobody® that

i) has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1 to 22, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

Aspect F-24: An amino acid sequence according to any of aspects F-1 to F-23, that essentially consists of a Nanobody® that

i) has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

Aspect F-25: An amino acid sequence according to any of aspects F-1 to F-24, that essentially consists of a humanized Nanobody®.

Aspect G-1: An amino acid sequence according to any of the preceding aspects, that in addition to the at least one binding site for binding formed by the CDR sequences, contains one or more further binding sites for binding against another antigen, protein or target.

Aspect H-1: Nanobody® that is directed against and/or that can specifically bind to Dkk-1.

Aspect H-2: Nanobody® according to aspect H-1, that can specifically bind Dkk-1 but not Dkk-2, Dkk-3 or Dkk-4.

Aspect H-3: Nanobody® according to aspect H-1, that can specifically bind Dkk-1, Dkk-2 and Dkk-4 but not Dkk-3; or that can specifically bind Dkk-1 and Dkk-4 but not Dkk-2 or Dkk-3.

Aspect H-4: Nanobody® according to any of aspects H-1 to H-3, that inhibits and/or blocks binding of LRP5/6 to Dkk-1.

Aspect H-5: Nanobody® according to any of aspects H-1 to H-4, that competes with LRP5/6 for binding to Dkk-1.

Aspect H-6: Nanobody® according to any of aspects H-1 to H-5, that binds to the LRP5/6 binding site on Dkk-1.

Aspect H-7: Nanobody® according to any of aspects H-1 to H-6, that binds to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 on Dkk-1.

Aspect H-8: Nanobody® according to any of aspects H-1 to H-7, that binds to at least one of amino acid residues R242 or H210 on Dkk-1.

Aspect H-9: Nanobody® according to any of aspects H-1 to H-8, that inhibits and/or blocks binding of BHQ880 to Dkk-1.

Aspect H-10: Nanobody® according to any of aspects H-1 to H-9, that competes with BHQ880 for binding to Dkk-1.

Aspect H-11: Nanobody® according to any of aspects H-1 to H-10, that binds to the BHQ880 binding site on Dkk-1.

Aspect H-12: Nanobody® according to any of aspects H-1 to H-11, that inhibits and/or blocks binding of RH2-18 to Dkk-1.

Aspect H-13: Nanobody® according to any of aspects H-1 to H-12, that competes with RH2-18 for binding to Dkk-1.

Aspect H-14: Nanobody® according to any of aspects H-1 to H-13, that binds to the RH2-18 binding site on Dkk-1.

Aspect H-15: Nanobody® according to any of aspects H-1 to H-14, that inhibits and/or blocks binding of 11H10 to Dkk-1.

Aspect H-16: Nanobody® according to any of aspects H-1 to H-15, that competes with 11H10 for binding to Dkk-1.

Aspect H-17: Nanobody® according to any of aspects H-1 to H-16, that binds to the 11H10 binding site on Dkk-1.

Aspect H-18: Nanobody® according to any of aspects H-1 to H-17, that inhibits and/or blocks binding of Kremen to Dkk-1.

Aspect H-19: Nanobody® according to any of aspects H-1 to H-18, that competes with Kremen for binding to Dkk-1.

Aspect H-20: Nanobody® according to any of aspects H-1 to H-19, that binds to the Kremen binding site on Dkk-1.

Aspect H-21: Nanobody® according to any of aspects H-1 to H-20, that binds to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1.

Aspect H-22: Nanobody® according to any of aspects H-1 to H-21, that binds to at least one of amino acid residues R197 or K232 on Dkk-1.

Aspect H-23: Nanobody® according to any of aspects H-1 to H-22, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway.

Aspect H-24: Nanobody® according to aspects H-23, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than BHQ880.

Aspect H-25: Nanobody® according to aspects H-23, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than RH2-18.

Aspect H-26: Nanobody® according to aspects H-23, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than 11H10.

Aspect H-27: Nanobody® according to any of aspects H-1 to H-26, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction.

Aspect H-28: Nanobody® according to aspects H-27, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than BHQ880.

Aspect H-29: Nanobody® according to aspects H-27, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than RH2-18.

Aspect H-30: Nanobody® according to aspects H-27, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than 11H10.

Aspect H-31: Nanobody® according to any of aspects H-1 to H-30, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation.

Aspect H-32: Nanobody® according to aspects H-31, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than BHQ880.

Aspect H-33: Nanobody® according to aspects H-31, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than RH2-18.

Aspect H-34: Nanobody® according to aspects H-31, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than 11H10.

Aspect H-35: Nanobody® according to any of aspects H-1 to H-34, that increases bone mass in a subject.

Aspect H-36: Nanobody® according to aspect H-35, that increases bone mass in a subject equally or better than BHQ880.

Aspect H-37: Nanobody® according to aspect H-35, that increases bone mass in a subject equally or better than RH2-18.

Aspect H-38: Nanobody® according to aspect H-35, that increases bone mass in a subject equally or better than 11H10.

Aspect H-39: Nanobody® according to any of aspects H-1 to H-38, that reduces bone lesions.

Aspect H-40: Nanobody® according to aspect H-39, that reduces bone lesions equally or better than BHQ880.

Aspect H-41: Nanobody® according to aspect H-39, that reduces bone lesions equally or better than RH2-18.

Aspect H-42: Nanobody® according to aspect H-39, that reduces bone lesions equally or better than 11H10.

Aspect H-43: Nanobody® according to any of aspects H-1 to H-42, that reduces tumor burden.

Aspect H-44: Nanobody® according to aspect H-43, that reduces tumor burden equally or better than BHQ880.

Aspect H-45: Nanobody® according to aspect H-43, that reduces tumor burden equally or better than RH2-18.

Aspect H-46: Nanobody® according to aspect H-43, that reduces tumor burden equally or better than 11H10.

Aspect H-47: Nanobody® according to any of aspects H-1 to H-46, that modulates the inhibitory effect of Dkk-1 on Wnt signaling.

Aspect H-48: Nanobody® according to aspect H-47, wherein said amino acid sequence modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as BHQ880.

Aspect H-49: Nanobody® according to aspect H-47, wherein said amino acid sequence modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as RH2-18.

Aspect H-50: Nanobody® according to aspect H-47, wherein said amino acid sequence modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as 11H10

Aspect H-51: Nanobody® according to any of aspects H-1 to H-50, that is in essentially isolated form.

Aspect H-52: Nanobody® according to any of aspects H-1 to H-51, that can specifically bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/litre or less, and preferably 10^{-7} to 10^{-12} moles/litre or less and more preferably 10^{-8} to 10^{-12} moles/litre.

Aspect H-53: Nanobody® according to any of aspects H-1 to H-52, that can specifically bind to Dkk-1 with a rate of association (k_{on}-rate) of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$.

Aspect H-54: Nanobody® according to any of aspects H-1 to H-53, that can specifically bind to Dkk-1 with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Aspect H-55: Nanobody® according to any of aspects H-1 to H-54, that can specifically bind to Dkk-1 with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

Aspect H-56: Nanobody® according to any of aspects H-1 to H-55, that is a naturally occurring Nanobody® (from any suitable species) or a synthetic or semi-synthetic Nanobody®.

Aspect H-57: Nanobody® according to any of aspects to H-1 to H-56, that is a V_{HH} sequence, a partially humanized V_{HH} sequence, a fully humanized V_{HH} sequence, a camelized heavy chain variable domain or a Nanobody® that has been obtained by techniques such as affinity maturation.

Aspect H-58: Nanobody® according to any of aspects H-1 to H-57, that

i) has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1 to 22, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

Aspect H-59: Nanobody® according to any of aspects H-1 to H-58, that

i) has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

Aspect H-60: Nanobody® according to any of aspects H-1 to H-59, in which:

- CDR1 is chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 335-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

and/or

- CDR2 is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

and/or

- CDR3 is chosen from the group consisting of:

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379.

Aspect H-61: Nanobody® according to any of aspects H-1 to H-60, in which:

- CDR1 is chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 335-543;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 335-543;

and

- CDR2 is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 753-961;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;

and

- CDR3 is chosen from the group consisting of:

g) the amino acid sequences of SEQ ID NO's: 1171-1379;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379.

Aspect H-62: Nanobody® according to any of aspects H-1 to H-61, in which the CDR sequences have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797.

Aspect H-63: Nanobody® according to any of aspects H-1 to H-62, which is a partially humanized Nanobody®.

Aspect H-64: Nanobody® according to any of aspects H-1 to H-63, which is a fully humanized Nanobody®.

Aspect H-65: Nanobody® according to any of aspects H-1 to H-64, that is chosen from the group consisting of SEQ ID NO's: 1589-1797 or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797.

Aspect H-66: Nanobody® according to any of aspects H-1 to H-65, which is a humanized Nanobody® that is chosen from the group consisting of SEQ ID NO's: 1798-1816 or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with at least one of the amino acid sequences of SEQ ID NO's: 1798-1816.

Aspect H-67: Nanobody® according to any of aspects H-1 to H-66, that is chosen from the group consisting of SEQ ID NO's: 1589-1797 or from the group consisting of SEQ ID NO's: 1798-1816.

Aspect H-68: Nanobody® directed against Dkk-1 that cross-blocks the binding of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 to Dkk-1.

Aspect H-69: Nanobody® directed against Dkk-1 that is cross-blocked from binding to Dkk-1 by at least one of the amino acid sequences of SEQ ID NO's: 1589-1797.

Aspect H-70: Nanobody® according to any of aspects H-68 or H-69 wherein the ability of said Nanobody® to cross-block or to be cross-blocked is detected in a Biacore assay.

Aspect H-71: Nanobody® according to any of aspects H-68 or H-69 wherein the ability of said Nanobody® to cross-block or to be cross-blocked is detected in an ELISA assay.

Aspect K-1: Polypeptide that comprises or essentially consists of one or more amino acid sequences according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 and/or one or more Nanobodies® according to any of aspects H-1 to H-71, and optionally further comprises one or more peptidic linkers.

Aspect K-2: Polypeptide according to aspect K-1, in which said one or more binding units are immunoglobulin sequences.

Aspect K-3: Polypeptide according to any of aspects K-1 or K-2, in which said one or more other groups, residues, moieties or binding units are chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s, amino acid sequences that are suitable for use as a dAb, or Nanobodies®.

Aspect K-4: Polypeptide according to any of aspects K-1 to K-3, in which said one or more amino acid sequences of the invention are immunoglobulin sequences.

Aspect K-5: Polypeptide according to any of aspects K-1 to K-4, in which said one or more amino acid sequences of the invention are chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s, amino acid sequences that are suitable for use as a dAb, or Nanobodies®.

Aspect K-6: Polypeptide according to any of aspects K-1 to K-5, that comprises or essentially consists of one or more Nanobodies® according to any of aspects H-1 to H-71 and in which said one or more other binding units are Nanobodies®.

Aspect K-7: Polypeptide according to any of aspects K-1 to K-6, which is a multivalent construct.

Aspect K-8: Polypeptide according to any of aspects K-1 to K-7, which is a multiparatopic construct.

Aspect K-9: Polypeptide according to any of aspects K-1 to K-7, which is a multispecific construct.

Aspect K-10: Polypeptide according to any of aspects K-1 to K-9, which has an increased half-life, compared to the corresponding amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 per se or Nanobody® according to any of aspects H-1 to H-71 per se, respectively.

Aspect K-11: Polypeptide according to aspect K-10, in which said one or more other binding units provide the polypeptide with increased half-life, compared to the corresponding amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 per se or Nanobody® according to any of aspects H-1 to H-71 per se, respectively.

Aspect K-12: Polypeptide according to aspect K-10 or K-11, in which said one or more other binding units that provide the polypeptide with increased half-life is chosen from the group consisting of serum proteins or fragments thereof, binding units that can bind to serum proteins, an Fc portion, and small proteins or peptides that can bind to serum proteins.

Aspect K-13: Polypeptide according to any of aspects K-10 to K-12, in which said one or more other binding units that provide the polypeptide with increased half-life is chosen from the group consisting of human serum albumin or fragments thereof.

Aspect K-14: Polypeptide according to any of aspect K-10 to K-13, in which said one or more other binding units that provides the polypeptide with increased half-life are chosen from the group consisting of binding units that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

Aspect K-15: Polypeptide according to any of aspects K-10 to K-14, in which said one or more other binding units that provides the polypeptide with increased half-life are chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s, amino acid sequences that are suitable for use as a dAb, or Nanobodies® that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

Aspect K-16: Polypeptide according to aspect K-10 to K-15, in which said one or more other binding units that provides the polypeptide with increased half-life is a Nanobody® that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

Aspect K-17: Polypeptide according to any of aspects K-10 to K-16, that has a serum half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at

least 10 times or more than 20 times, greater than the half-life of the corresponding amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 per se or Nanobody® according to any of aspects H-1 to H-71 per se, respectively.

Aspect K-18: Polypeptide according to any of aspects K-10 to K-17, that has a serum half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 per se or Nanobody® according to any of aspects H-1 to H-71 per se, respectively.

Aspect K-19: Polypeptide according to any of aspects K-1 to K-18, that has a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more; for example, of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

Aspect L-1: Compound or construct, that comprises or essentially consists of one or more amino acid sequences according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 and/or one or more Nanobodies® according to any of aspects H-1 to H-71, and optionally further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers.

Aspect L-2: Compound or construct according to aspects L-1, in which said one or more other groups, residues, moieties or binding units are amino acid sequences.

Aspect L-3: Compound or construct according to aspect L-1 or L-2, in which said one or more linkers, if present, are one or more amino acid sequences.

Aspect L-4: Compound or construct according to any of aspects L-1 to L-3, in which said one or more other groups, residues, moieties or binding units are immunoglobulin sequences.

Aspect L-5: Compound or construct according to any of aspects L-1 to L-4, in which said one or more other groups, residues, moieties or binding units are chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable

for use as a single domain antibody, "dAb"'s, amino acid sequences that are suitable for use as a dAb, or Nanobodies®.

Aspect L-6: Compound or construct according to any of aspects L-1 to L-5, in which said one or more amino acid sequences of the invention are immunoglobulin sequences.

Aspect L-7: Compound or construct according to any of aspects L-1 to L-6, in which said one or more amino acid sequences of the invention are chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s, amino acid sequences that are suitable for use as a dAb, or Nanobodies®.

Aspect L-8: Compound or construct, that comprises or essentially consists of one or more Nanobodies® according to any of aspects H-1 to H-71 and in which said one or more other groups, residues, moieties or binding units are Nanobodies®.

Aspect L-9: Compound or construct according to any of aspects L-1 to L-8, which is a multivalent construct.

Aspect L-10: Compound or construct according to any of aspects L-1 to L-9, which is a multiparatopic construct.

Aspect L-11: Compound or construct according to aspect L-10, which is a biparatopic or triparatpic construct.

Aspect L-12: Compound or construct according to any of aspects L-10 and/or L-11, which comprises at least one amino acid sequence directed against a first antigenic determinant, epitope, part or domain of Dkk-1 and at least one amino acid sequence directed against a second antigenic determinant, epitope, part or domain of Dkk-1 different from the first antigenic determinant, epitope, part or domain.

Aspect L-13: Biparatopic compound or construct according to aspect L-12, which is capable of simultaneously binding to said first antigenic determinant, epitope, part or domain of Dkk-1 and to said second antigenic determinant, epitope, part or domain of Dkk-1.

Aspect L-14: Compound or construct according to any of aspects L-11 to L-13, which combines two or more different modes of action each mediated by one of its binding units, wherein each binding unit binds at a different binding site of Dkk-1.

Aspect L-15: Compound or construct according to any of aspects L-11 to L-14, wherein said compound or construct competes with LRP5/6 for binding to Dkk-1.

Aspect L-16: Compound or construct according to any of aspects L-11 to L-15, wherein said compound or construct inhibits and/or blocks binding of LRP5/6 to Dkk-1.

Aspect L-17: Compound or construct according to any of aspects L-11 to L-16, wherein said compound or construct is directed against the LRP5/6 binding site on Dkk-1.

Aspect L-18: Compound or construct according to any of aspects L-11 to L-17, wherein said compound or construct specifically binds to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1.

Aspect L-19: Compound or construct according to any of aspects L-11 to L-18, wherein said compound or construct specifically binds to at least one of amino acid residues R242 or H210 of Dkk-1.

Aspect L-20: Compound or construct according to any of aspects L-11 to L-19, wherein said compound or construct competes with BHQ880 for binding to Dkk-1.

Aspect L-21: Compound or construct according to any of aspects L-11 to L-20, wherein said compound or construct inhibits and/or blocks binding of BHQ880 to Dkk-1.

Aspect L-22: Compound or construct according to any of aspects L-11 to L-21, wherein said compound or construct is directed against the BHQ880 binding site on Dkk-1.

Aspect L-23: Compound or construct according to any of aspects L-11 to L-22, wherein said compound or construct modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as BHQ880.

Aspect L-24: Compound or construct according to any of aspects L-11 to L-23, wherein said compound or construct competes with RH2-18 for binding to Dkk-1.

Aspect L-25: Compound or construct according to any of aspects L-11 to L-24, wherein said compound or construct inhibits and/or blocks binding of RH2-18 to Dkk-1.

Aspect L-26: Compound or construct according to any of aspects L-11 to L-25, wherein said compound or construct is directed against the RH2-18 binding site on Dkk-1.

Aspect L-27: Compound or construct according to any of aspects L-11 to L-26, wherein said compound or construct modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as RH2-18.

Aspect L-28: Compound or construct according to any of aspects L-11 to L-27, wherein said compound or construct competes with 11H10 for binding to Dkk-1.

Aspect L-29: Compound or construct according to any of aspects L-11 to L-28, wherein said compound or construct inhibits and/or blocks binding of 11H10 to Dkk-1.

Aspect L-30: Compound or construct according to any of aspects L-11 to L-29, wherein said compound or construct is directed against the 11H10 binding site on Dkk-1.

Aspect L-31: Compound or construct according to any of aspects L-11 to L-30, wherein said compound or construct modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as 11H10.

Aspect L-32: Compound or construct according to any of aspects L-11 to L-31, wherein said compound or construct competes with Kremen for binding to Dkk-1.

Aspect L-33: Compound or construct according to any of aspects L-11 to L-32, wherein said compound or construct inhibits and/or blocks binding of Kremen to Dkk-1.

Aspect L-34: Compound or construct according to any of aspects L-11 to L-33, wherein said compound or construct is directed against the Kremen binding site on Dkk-1.

Aspect L-35: Compound or construct according to any of aspects L-11 to L-34, wherein said compound or construct specifically binds to at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1.

Aspect L-36: Compound or construct according to any of aspects L-11 to L-35, wherein said compound or construct specifically binds to at least one of amino acid residues R197 or K232 of Dkk-1.

Aspect L-37: Compound or construct according to any of aspects L-11 to L-36, wherein said compound or construct is directed against the LRP5/6 binding site on Dkk-1 and against the Kremen binding site on Dkk-1.

Aspect L-38: Compound or construct according to any of aspects L-11 to L-37, wherein said compound or construct competes with LRP5/6 and Kremen for binding to Dkk-1.

Aspect L-39: Compound or construct according to any of aspects L-11 to L-38, wherein said compound or construct inhibits and/or blocks binding of LRP5/6 and Kremen to Dkk-1.

Aspect L-40: Compound or construct according to any of aspects L-11 to L-39, which specifically binds the LRP5/6 binding site on Dkk-1 and to the Kremen binding site on Dkk-1.

Aspect L-41: Compound or construct according to any of aspects L-11 to L-40, which specifically binds to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1.

Aspect L-42: Compound or construct according to any of aspects L-11 to L-41, which specifically binds to at least one of amino acid residues R242 or H210 of Dkk-1.

Aspect L-43: Compound or construct according to any of aspects L-11 to L-42, which specifically binds to at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1.

Aspect L-44: Compound or construct according to any of aspects L-11 to L-43, which specifically binds to at least one of amino acid residues R197 or K232 of Dkk-1.

Aspect L-45: Biparatopic compound or construct according to any of aspects L-11 to L-44, which can simultaneously bind the LRP5/6 binding site on Dkk-1 and to the Kremen binding site on Dkk-1.

Aspect L-46: Compound or construct according to any of aspects L-11 to L-45, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway.

Aspect L-47: Compound or construct according to aspects L-46, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than BHQ880.

Aspect L-48: Compound or construct according to aspects L-46, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than RH2-18.

Aspect L-49: Compound or construct according to aspects L-46, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than 11H10.

Aspect L-50: Compound or construct according to any of aspects L-11 to L-49, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction.

Aspect L-51: Compound or construct according to aspects L-50, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than BHQ880.

Aspect L-52: Compound or construct according to aspects L-50, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than RH2-18.

Aspect L-53: Compound or construct according to aspects L-50, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than 11H10.

Aspect L-54: Compound or construct according to any of aspects L-11 to L-53, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation.

Aspect L-55: Compound or construct according to aspects L-54, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than BHQ880.

Aspect L-56: Compound or construct according to aspects L-54, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than RH2-18.

Aspect L-57: Compound or construct according to aspects L-54, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than 11H10.

Aspect L-58: Compound or construct according to any of aspects L-11 to L-53, that increases bone mass in a subject.

Aspect L-59: Compound or construct according to aspect L-58, that increases bone mass in a subject equally or better than BHQ880.

Aspect L-60: Compound or construct according to aspect L-58, that increases bone mass in a subject equally or better than RH2-18.

Aspect L-61: Compound or construct according to aspect L-58, that increases bone mass in a subject equally or better than 11H10.

Aspect L-62: Compound or construct according to any of aspects L-11 to L-61, that reduces bone lesions.

Aspect L-63: Compound or construct according to aspect L-62, that reduces bone lesions equally or better than BHQ880.

Aspect L-64: Compound or construct according to aspect L-62, that reduces bone lesions equally or better than RH2-18.

Aspect L-65: Compound or construct according to aspect L-62, that reduces bone lesions equally or better than 11H10.

Aspect L-66: Compound or construct according to any of aspects L-11 to L-65, that reduces tumor burden.

Aspect L-67: Compound or construct according to aspect L-66, that reduces tumor burden equally or better than BHQ880.

Aspect L-68: Compound or construct according to aspect L-66, that reduces tumor burden equally or better than RH2-18.

Aspect L-69: Compound or construct according to aspect L-66, that reduces tumor burden equally or better than 11H10.

Aspect L-70: Compound or construct according to any of aspects L-11 to L-69, which is a multispecific construct.

Aspect L-71: Compound or construct that comprises or that is chosen from the group consisting of SEQ ID NO's: 1817-1820 or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with at least one of the amino acid sequences of SEQ ID NO's: 1817-1820.

Aspect L-72: Compound or construct according to any of aspects L-1 to L-71, which has an increased half-life, compared to the corresponding amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 per se or Nanobody® according to any of aspects H-1 to H-71 per se, respectively.

Aspect L-73: Compound or construct according to aspect L-1 to L-72, in which said one or more other groups, residues, moieties or binding units provide the compound or construct with increased half-life, compared to the corresponding amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 per se or Nanobody® according to any of aspects H-1 to H-71 per se, respectively.

Aspect L-74: Compound or construct according to aspect L-73, in which said one or more other groups, residues, moieties or binding units that provide the compound or construct with increased half-life is chosen from the group consisting of serum proteins or fragments thereof, binding units that can bind to serum proteins, an Fc portion, and small proteins or peptides that can bind to serum proteins.

Aspect L-75: Compound or construct according to aspect L-73 or L-74, in which said one or more other groups, residues, moieties or binding units that provide the compound or construct with increased half-life is chosen from the group consisting of human serum albumin or fragments thereof.

Aspect L-76: Compound or construct according to any of aspects L-73 to L-75, in which said one or more other groups, residues, moieties or binding units that provides the compound or construct with increased half-life are chosen from the group consisting of binding units that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

Aspect L-77: Compound or construct according to any of aspects L-73 to L-75, in which said one or more other groups, residues, moieties or binding units that provides the compound or construct with increased half-life are chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s , amino acid sequences that are suitable for use as a dAb, or Nanobodies® that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

Aspect L-78: Compound or construct according to any of aspects L-73 to L-75, in which said one or more other groups, residues, moieties or binding units that provides the compound or construct with increased half-life is a Nanobody® that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

Aspect L-79: Compound or construct according to any of aspects L-73 to L-78, that has a serum half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 per se or Nanobody® according to any of aspects H-1 to H-71 per se, respectively.

Aspect L-80: Compound or construct according to any of aspects L-73 to L-79, that has a serum half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than

24, 48 or 72 hours, compared to the corresponding amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 per se or Nanobody® according to any of aspects H-1 to H-71 per se, respectively.

Aspect L-81: Compound or construct according to any of aspects L-73 to L-80, that has a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more; for example, of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

Aspect L-82: Monovalent construct, comprising or essentially consisting of one amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 and/or one Nanobody® according to any of aspects H-1 to H-71.

Aspect L-83: Monovalent construct according to aspect L-82, in which said amino acid sequence of the invention is chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s, amino acid sequences that are suitable for use as a dAb, or Nanobodies®.

Aspect L-84: Monovalent construct, comprising or essentially consisting of one Nanobody® according to any of aspects H-1 to H-71.

Aspect L-85: Monovalent construct, that is chosen from the group consisting of SEQ ID NO's: 1589-1797 or from the group consisting of amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with at least one of the amino acid sequences of SEQ ID NO's: 1589-1797.

Aspect L-86: Use of a monovalent construct according to any of aspects L-82 to L 85, in preparing a multivalent compound or construct according to any of aspects L-1 to L-85.

Aspect L-87: Use of a monovalent construct according to aspect L-86, in preparing a multiparatopic construct such as a biparatopic construct.

Aspect L-88: Use of a monovalent construct according to any of aspects L-86 or L-87, wherein the monovalent construct is used as a binding domain or binding unit in preparing a multivalent construct comprising two or more binding units.

Aspect L-89: Use of a monovalent construct according to any of aspects L-86 to L-88, in preparing a multivalent construct that exhibits intramolecular binding compared to intermolecular binding.

Aspect L-90: Use of a monovalent construct according to any of aspects L-86 to L-89, as a binding domain or binding unit in preparing a multivalent construct, wherein the binding domains or binding units are linked via a linker such that the multivalent construct preferably exhibits intramolecular binding compared to intermolecular binding.

Aspect L-91: Use of a monovalent construct according to any of aspects L-86 to L-90, wherein the monovalent construct is directed against the LRP5/6 binding site on Dkk-1 and/or is capable of competing with LRP5/6 for binding to Dkk-1.

Aspect L-92: Use of a monovalent construct according to any of aspects L-86 to L-91, wherein the monovalent construct is directed against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1.

Aspect L-93: Use of a monovalent construct according to any of aspects L-86 to L-92, wherein the monovalent construct is directed against at least one of amino acid residues R242 or H210 of Dkk-1.

Aspect L-94: Use of a monovalent construct according to any of aspects L-86 to L-93, wherein the monovalent construct is directed against the BHQ880 binding site on Dkk-1 and/or is capable of competing with BHQ880 for binding to Dkk-1.

Aspect L-95: Use of a monovalent construct according to any of aspects L-86 to L-94, wherein the monovalent construct is directed against the BHQ880 binding site on Dkk-1 and/or is capable of competing with RH2-18 for binding to Dkk-1.

Aspect L-96: Use of a monovalent construct according to any of aspects L-86 to L-95, wherein the monovalent construct is directed against the BHQ880 binding site on Dkk-1 and/or is capable of competing with 11H10 for binding to Dkk-1.

Aspect L-97: Use of a monovalent construct according to any of aspects L-86 to L-96, wherein the monovalent construct is directed against the Kremen binding site on Dkk-1 and/or is capable of competing with Kremen for binding Dkk-1.

Aspect L-98: Use of a monovalent construct according to any of aspects L-86 to L-97, wherein the monovalent construct is directed against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1.

Aspect L-99: Use of a monovalent construct according to any of aspects L-86 to L-98, wherein the monovalent construct is directed against at least one of amino acid residues R197 or K232 of Dkk-1.

Aspect L-100: Use of two monovalent constructs according to any of aspects L-86 to L-99, wherein a first monovalent construct is directed against the LRP5/6 binding site on Dkk-1 (and in particular against at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, and more in particular against at least one of amino acid residues R242 or H210 of Dkk-1) and/or is capable of competing with LRP5/6 for binding to Dkk-1 and wherein the second monovalent construct is directed against the Kremen binding site on Dkk-1 (and in particular against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, and more in particular against at least one of amino acid residues R197 or K232 of Dkk-1) and/or is capable of competing with Kremen for binding to Dkk-1.

Aspect L-101: Use of two monovalent constructs according to any of aspects L-86 to L-100, wherein a first monovalent construct is directed against the BHQ880 binding site on Dkk-1 and/or is capable of competing with BHQ880 for binding to Dkk-1 and wherein the second monovalent construct is directed against the Kremen binding site on Dkk-1 (and in particular against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, and more in particular against at least one of amino acid residues R197 or K232 of Dkk-1) and/or is capable of competing with Kremen for binding to Dkk-1.

Aspect L-102: Use of two monovalent constructs according to any of aspects L-86 to L-100, wherein a first monovalent construct is directed against the RH2-18 binding site on Dkk-1 and/or is capable of competing with RH2-18 for binding to Dkk-1 and wherein the second monovalent construct is directed against the Kremen binding site on Dkk-1 (and in particular against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, and more in particular against at least one of amino acid residues R197 or K232 of Dkk-1) and/or is capable of competing with Kremen for binding to Dkk-1.

Aspect L-103: Use of two monovalent constructs according to any of aspects L-86 to L-100, wherein a first monovalent construct is directed against the 11H10 binding site on Dkk-1 and/or is capable of competing with 11H10 for binding to Dkk-1 and wherein the second monovalent construct is directed against the Kremen binding site on Dkk-1 (and in particular against at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, and more in particular against at least one of amino acid residues

R197 or K232 of Dkk-1) and/or is capable of competing with Kremen for binding to Dkk-1.

Aspect M-1: Nucleic acid or nucleotide sequence, that encodes an amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, a Nanobody® according to any of aspects H-1 to H-71, a compound or construct according to any of aspects that is such that it can be obtained by expression of a nucleic acid or nucleotide sequence encoding the same, or a monovalent construct according to any of aspects L-82 to L-85.

Aspect M-2: Nucleic acid or nucleotide sequence according to aspect M-1, that is in the form of a genetic construct.

Aspect M-3: Use of a nucleic acid or nucleotide sequence according to aspect M-1, that encodes a monovalent construct according to any of aspects L-82 to L-85, for the preparation of a genetic construct that encodes a multivalent construct according to any of aspects L-1 to L-81.

Aspect M-4: Use of a nucleic acid or nucleotide sequence according to aspect M-2, wherein the genetic construct encodes a multiparatopic (such as a biparatopic) construct.

Aspect N-1: Host or host cell that expresses, or that under suitable circumstances is capable of expressing, an amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, a Nanobody® according to any of aspects H-1 to H-71, a polypeptide according to any of aspects K-1 to K-19, a compound or construct according to any of aspects L-1 to L-81 that is such that it can be obtained by expression of a nucleic acid or nucleotide sequence encoding the same, or a monovalent construct according to any of aspects L-82 to L-85; and/or that comprises a nucleic acid or nucleotide sequence according to aspect M-1 or a genetic construct according to aspect M-2.

Aspect O-1: Composition comprising at least one amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, Nanobody® according to any of aspects H-1 to H-71, polypeptide according to any of aspects K-1 to K-19, compound or construct according to any of aspects L-1 to L-81, monovalent construct according to any of aspects L-82 to L-85, or nucleic acid or nucleotide sequence according to aspects M-1 or M-2.

Aspect O-2: Composition according to aspect O-1, which is a pharmaceutical composition.

Aspect O-3: Composition according to aspect O-2, which is a pharmaceutical composition, that further comprises at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and that optionally comprises one or more further pharmaceutically active polypeptides and/or compounds.

Aspect P-1: Method for producing an amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, a Nanobody® according to any of aspects H-1 to H-71, a polypeptide according to any of aspects K-1 to K-19, a compound or construct according to any of aspects L-1 to L-81 that is such that it can be obtained by expression of a nucleic acid or nucleotide sequence encoding the same, or a monovalent construct according to any of aspects L-82 to L-85, said method at least comprising the steps of:

a) expressing, in a suitable host cell or host organism or in another suitable expression system, a nucleic acid or nucleotide sequence according to aspect M-1, or a genetic construct according to aspect M-2;

optionally followed by:

b) isolating and/or purifying the amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, a Nanobody® according to any of aspects H-1 to H-71, a polypeptide according to any of aspects K-1 to K-19, a compound or construct according to any of aspects L-1 to L-81, or a monovalent construct according to any of aspects L-82 to L-85 thus obtained.

Aspect P-2: Method for producing an amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, a Nanobody® according to any of aspects H-1 to H-71, a polypeptide according to any of aspects K-1 to K-19, a compound or construct according to any of aspects L-1 to L-81 that is such that it can be obtained by expression of a nucleic acid or nucleotide sequence encoding the same, or a monovalent construct according to any of aspects L-82 to L-85, said method at least comprising the steps of:

a) cultivating and/or maintaining a host or host cell according to aspect N-1 under conditions that are such that said host or host cell expresses and/or produces at least one amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, Nanobody® according to any of aspects H-1 to H-71, a polypeptide according to any of aspects K-1 to K-

19, a compound or construct according to any of aspects L-1 to L-81, or monovalent construct according to any of aspects L-82 to L-85;

optionally followed by:

b) isolating and/or purifying the amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, Nanobody® according to any of aspects H-1 to H-71, a polypeptide according to any of aspects K-1 to K-19, a compound or construct according to any of aspects L-1 to L-81, or monovalent construct according to any of aspects L-82 to L-85 thus obtained.

Aspect P-3: Method for preparing and/or generating a multiparatopic (such as e.g. biparatopic, triparatopic, etc.) construct according to any of aspects L-11 to L-81, said method comprising at least the steps of:

a) providing a nucleic acid sequence according to aspect M-1, encoding a first Dkk-1 binding amino acid sequence, fused to a set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode a second amino acid sequence that can bind to and/or has affinity for an antigenic determinant on Dkk-1 different from the antigenic determinant recognized by the first Dkk-1 binding amino acid sequence;

and

c) isolating the nucleic acid sequence encoding a Dkk-1 binding amino acid sequence fused to the nucleic acid sequence obtained in b), followed by expressing the encoded construct.

Aspect P-4: Method for preparing and/or generating a biparatopic construct according to any of aspects L-11 to L-81, said method comprising at least the steps of:

a) providing a set, collection or library of nucleic acid sequences, in which each nucleic acid sequence in said set, collection or library encodes a fusion protein that comprises a first amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1 that is fused (optionally via a linker sequence) to a second amino acid sequence, in which essentially each second amino acid sequence (or most of these) is a different member of a set, collection or library of different amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has

affinity for a second antigenic determinant, part, domain or epitope on Dkk-1 different from the first antigenic determinant, part, domain or epitope on Dkk-1; and

c) isolating the nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on Dkk-1 different from the first antigenic determinant, part, domain or epitope on Dkk-1, obtained in b), optionally followed by expressing the encoded amino acid sequence.

Aspect P-5: Method according to aspect P-4, wherein the first amino acid is also encoded by a set, collection or library of nucleic acid sequences and wherein, in step b), said set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the first antigenic determinant, part, domain or epitope on Dkk-1

Aspect P-6: Method according to aspect P-5, wherein the screening in step b) is performed in a single step.

Aspect P-7: Method according to aspect P-5, wherein the screening in step b) is performed in subsequent steps.

Aspect P-8: Method according to any of aspects P-4 to P-7, wherein the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or (ii) it competes with LRP5/6 for binding to Dkk-1.

Aspect P-9: Method according to any of aspects P-4 to P-7, wherein the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or (ii) it competes with BHQ880 for binding to Dkk-1.

Aspect P-10: Method according to any of aspects P-4 to P-7, wherein the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or (ii) it competes with RH2-18 for binding to Dkk-1.

Aspect P-11: Method according to any of aspects P-4 to P-7, wherein the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or (ii) it competes with 11H10 for binding to Dkk-1.

Aspect P-12: Method according to any of aspects P-4 to P-11, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that

encode (i) an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or (ii) an amino acid sequence that can compete with Kremen for binding to Dkk-1.

Aspect P-13: Method according to any of aspects P-4 to P-7, wherein the first amino acid sequence used in step a) is preferably such that (i) it can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1.

Aspect P-14: Method according to any of aspects P-4 to P-7 and P-13, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or (ii) an amino acid sequence that can compete with LRP5/6 for binding to Dkk-1.

Aspect P-15: Method according to any of aspects P-4 to P-7 and P-13, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or (ii) an amino acid sequence that can compete with BHQ880 for binding to Dkk-1.

Aspect P-16: Method according to any of aspects P-4 to P-7 and P-13, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or (ii) an amino acid sequence that can compete with RH2-18 for binding to Dkk-1.

Aspect P-17: Method according to any of aspects P-4 to P-7 and P-13, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode (i) an amino acid sequence that can bind to and/or has affinity for the 11H1 binding site on Dkk-1 and/or (ii) an amino acid sequence that can compete with 11H1 for binding to Dkk-1.

Aspect P-18: Method according to any of aspects P-4 to P-17, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that both

(i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or that can compete with Kremen for binding to Dkk-1 and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or that can compete with LRP5/6 for binding to Dkk-1.

Aspect P-19: Method according to any of aspects P-4 to P-17, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or that can compete with Kremen for binding to Dkk-1 and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or that can compete with BHQ880 for binding to Dkk-1.

Aspect P-20: Method according to any of aspects P-4 to P-17, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or that can compete with Kremen for binding to Dkk-1 and that also (ii) encode an amino acid sequence that can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or that can compete with RH2-18 for binding to Dkk-1.

Aspect P-21: Method according to any of aspects P-4 to P-17, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that both (i) encode an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or that can compete with Kremen for binding to Dkk-1 and that also (ii) encode an amino acid sequence that can bind to and/or has

affinity for the 11H10 binding site on Dkk-1 and/or that can compete with 11H10 for binding to Dkk-1.

Aspect P-22: Method according to any of aspects P-18 to P-21, wherein the screening in step b) is performed in a single step.

Aspect P-23: Method according to any of aspects P-18 to P-21, wherein the screening in step b) is performed in subsequent steps.

Aspect P-24: Method according to any of aspects P-18 to P-23, wherein the screening in step b) is performed in the presence of LRP5/6, BHQ880, RH2-18, 11H10 and/or Kremen.

Aspect P-25: Method for screen for suitable and/or optimal linker lengths for linking a first and a second amino acid sequence in a biparatopic constructs according to any of aspects 161 to 204, wherein said method comprises at least the steps of:

a) providing a set, collection or library of nucleic acid sequences, in which each nucleic acid sequence in said set, collection or library encodes a fusion protein that comprises a first amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1 that is fused via a linker sequence to a second amino acid sequence that has can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on Dkk-1 (which may be the same or different as the first antigenic determinant, part, domain or epitope on Dkk-1), in which essentially each nucleic acid sequence (or most of these) encodes a fusion protein with a different linker sequence so as to provide a set, collection or library encoding different fusion proteins;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on Dkk-1;

and

c) isolating the nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on Dkk-1, optionally followed by expressing the encoded amino acid sequence.

Aspect P-26: Method according to aspect P-25, wherein the first amino acid sequence is an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209,

H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or that can compete with LRP5/6 for binding to Dkk-1.

Aspect P-27: Method according to aspect P-25, wherein the first amino acid sequence is an amino acid sequence that can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or that can compete with BHQ880 for binding to Dkk-1.

Aspect P-28: Method according to aspect P-25, wherein the first amino acid sequence is an amino acid sequence that can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or that can compete with RH2-18 for binding to Dkk-1.

Aspect P-29: Method according to aspect P-25, wherein the first amino acid sequence is an amino acid sequence that can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or that can compete with 11H10 for binding to Dkk-1.

Aspect P-30: Method according to aspect P-25, wherein the second amino acid sequence is an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or that can compete with Kremen for binding to Dkk-1.

Aspect P-31: Method according to aspect P-25, wherein the first amino acid sequence is an amino acid sequence that can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or that can compete with LRP5/6 for binding to Dkk-1 and wherein the second amino acid sequence is an amino acid sequence that can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or that can compete with Kremen for binding to Dkk-1.

Aspect P-32: Method according to any of aspects P-25 to P-31, wherein the screening in step b) is performed in a single step.

Aspect P-33: Method according to any of aspects P-25 to P-314, wherein the screening in step b) is performed in subsequent steps.

Aspect P-34: Method according to any of aspects P-25 to P-33, wherein the screening in step b) is performed in the presence of LRP5/6, BHQ880, RH2-18, 11H10 and/or Kremen.

Aspect P-35: Method for preparing and/or generating biparatopic constructs according to any of aspects L-11 to L-81, said method comprising at least the steps of:

a) providing a set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for a set, collection or library of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1;

c) ligating said set, collection or library of nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 to another nucleic acid sequence that encodes an amino acid sequence that can bind to and/or has affinity for Dkk-1 (e.g. a nucleic acid sequence that encodes an amino acid sequence that competes with LRP5/6 for binding Dkk-1);

and

d) from the set, collection or library of nucleic acid sequences obtained in c), isolating the nucleic acid sequences encoding a biparatopic amino acid sequence that can bind to and/or has affinity for Dkk-1 (and e.g. further selecting for nucleic acid sequences that encode a biparatopic amino acid sequence that antagonizes with higher potency compared to the monovalent amino acid sequences), followed by expressing the encoded amino acid sequence.

Aspect P-36: Method for preparing and/or generating biparatopic constructs according to any of aspects, said method comprising at least the steps of:

a) providing a first set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said first set, collection or library of nucleic acid sequences for a nucleic acid sequence that encodes an amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1;

c) ligating the nucleic acid sequence encoding said amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1 obtained in b) to another set, collection or library of nucleic acid sequences encoding amino acid sequences to obtain a set, collection or library of nucleic acid sequences that encode fusion proteins;

d) screening said set, collection or library of nucleic acid sequences obtained in step c) for a nucleic acid sequence that encodes an amino acid sequence that can bind to and has affinity for a second antigenic determinant, part, domain or epitope on Dkk-1 which is the same or different from the first antigenic determinant, part, domain or epitope on Dkk-1;

and

e) isolating the nucleic acid sequence that encodes an amino acid sequence that can bind to and/or has affinity for the first and second antigenic determinant, part, domain or epitope on Dkk-1, optionally followed by expressing the encoded amino acid sequence.

Aspect P-37: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or (ii) competes with LRP5/6 for binding to Dkk-1.

Aspect P-38: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or (ii) competes with BHQ880 for binding to Dkk-1.

Aspect P-39: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or (ii) competes with RH2-18 for binding to Dkk-1.

Aspect P-40: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or (ii) competes with 11H10 for binding to Dkk-1.

Aspect P-41: Method according to aspect P-36, wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or (ii) that can compete with Kremen for binding to Dkk-1.

Aspect P-42: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues

R242 or H210 of Dkk-1) and/or (ii) competes with LRP5/6 for binding to Dkk-1 and wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or (ii) that can compete with Kremen for binding to Dkk-1 (or visa versa).

Aspect P-43: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or (ii) competes with BHQ880 for binding to Dkk-1 and wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or (ii) that can compete with Kremen for binding to Dkk-1 (or visa versa).

Aspect P-44: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or (ii) competes with RH2-18 for binding to Dkk-1 and wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or (ii) that can compete with Kremen for binding to Dkk-1 (or visa versa).

Aspect P-45: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or (ii) competes with 11H10 for binding to Dkk-1 and wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of

amino acid residues R197 or K232 of Dkk-1) and/or (ii) that can compete with Kremen for binding to Dkk-1 (or visa versa).

Aspect P-46: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1.

Aspect P-47: Method according to aspect P-36, wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or (ii) that can compete with LRP5/6 for binding to Dkk-1.

Aspect P-48: Method according to aspect P-36, wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or (ii) that can compete with BHQ880 for binding to Dkk-1.

Aspect P-49: Method according to aspect P-36, wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or (ii) that can compete with RH2-18 for binding to Dkk-1.

Aspect P-50: Method according to aspect P-36, wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or (ii) that can compete with 11H10 for binding to Dkk-1.

Aspect P-51: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1 and wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has

affinity for the LRP5/6 binding site on Dkk-1 (and in particular at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 of Dkk-1, more in particular at least one of amino acid residues R242 or H210 of Dkk-1) and/or (ii) that can compete with LRP5/6 for binding to Dkk-1.

Aspect P-52: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1 and wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the BHQ880 binding site on Dkk-1 and/or (ii) that can compete with BHQ880 for binding to Dkk-1.

Aspect P-53: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1 and wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the RH2-18 binding site on Dkk-1 and/or (ii) that can compete with RH2-18 for binding to Dkk-1.

Aspect P-54: Method according to aspect P-36, wherein in step b), the set, collection or library of nucleic acid sequences is screened nucleic acid sequences that encode a first amino acid sequence that (i) can bind to and/or has affinity for the Kremen binding site on Dkk-1 (and in particular at least one of amino acid residues R197, R209, K214, or K232 of Dkk-1, more in particular at least one of amino acid residues R197 or K232 of Dkk-1) and/or (ii) competes with Kremen for binding to Dkk-1 and wherein in step d), the set, collection or library of nucleic acid sequences is screened for nucleic acid sequences that encode a second amino acid sequence that (i) can bind to and/or has affinity for the 11H10 binding site on Dkk-1 and/or (ii) that can compete with 11H10 for binding to Dkk-1.

Aspect P-55: Method according to any of aspects P-51 to P-54, wherein the screening in steps b) and/or d) is performed in the presence of LRP5/6, BHQ880, RH2-18, 11H10 and/or Kremen.

Aspect P-56: Method for preparing and/or generating a biparatopic constructs according to any of aspects L-11 to L-81, said method comprising at least the steps of linking two or more monovalent amino acid sequences or monovalent construct according to any of aspects L-82 to L-85 and for example one or more linkers.

Aspect P-57: Method according to aspect P-56, comprising the steps of:

a) linking two or more nucleic acid sequences according to aspect M-1, encoding a monovalent construct according to any of aspects L-82 to L-85 (and also for example nucleic acids encoding one or more linkers and further one or more further elements of genetic constructs known per se) to obtain a genetic construct according to aspect M-2;

b) expressing, in a suitable host cell or host organism or in another suitable expression system, the genetic construct obtained in a)

optionally followed by:

c) isolating and/or purifying the biparatopic constructs according to any of aspects L-11 to L-81 thus obtained.

Aspect Q-1: Method for screening amino acid sequences directed against Dkk-1 that comprises at least the steps of:

a) providing a set, collection or library of nucleic acid sequences encoding amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for Dkk-1 and that is cross-blocked or is cross blocking a Nanobody® of the invention, e.g. one of SEQ ID NO: 1589-1797 (Table A-1), or a humanized Nanobody® of the invention, e.g. SEQ ID NO: [1798-1816 (see Table A-2), or a polypeptide or construct of the invention, e.g. SEQ ID NO: 1817-1820 (see Table A-3); and

c) isolating said nucleic acid sequence, followed by expressing said amino acid sequence.

Aspect R-1: Method for the prevention and/or treatment of at least one Dkk-1-associated diseases or disorders, said method comprising administering, to a subject in need

thereof, a pharmaceutically active amount of at least one amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, Nanobody® according to any of aspects H-1 to H-71, polypeptide according to any of aspects K-1 to K-19, compound or construct according to any of aspects L-1 to L-81, monovalent construct according to any of aspects L-82 to L-85; or composition according to aspect O-2 or O-3.

Aspect R-2: Method for the prevention and/or treatment of at least one disease or disorder that is associated with Dkk-1, with its biological or pharmacological activity, and/or with the biological pathways or (inhibition of) signaling in which Dkk-1 is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, Nanobody® according to any of aspects H-1 to H-71, polypeptide according to any of aspects K-1 to K-19, compound or construct according to any of aspects L-1 to L-81, monovalent construct according to any of aspects L-82 to L-85; or composition according to aspect O-2 or O-3.

Aspect R-3: Method for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering, to a subject in need thereof, at least one amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, Nanobody® according to any of aspects H-1 to H-71, polypeptide according to any of aspects K-1 to K-19, compound or construct according to any of aspects L-1 to L-81, monovalent construct according to any of aspects L-82 to L-85; or composition according to aspect O-2 or O-3, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one at least one amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, Nanobody® according to any of aspects H-1 to H-71, polypeptide according to any of aspects K-1 to K-19, compound or construct according to any of aspects L-1 to L-81, monovalent construct according to any of aspects L-82 to L-85; or composition according to aspect O-2 or O-3.

Aspect R-4: Method for immunotherapy, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, Nanobody® according to any of aspects H-1 to H-71, polypeptide according to any of aspects K-1 to K-19, compound or construct according to any of

aspects L-1 to L-81, monovalent construct according to any of aspects L-82 to L-85; or composition according to aspect O-2 or O-3.

Aspect R-5: Use of an amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, a Nanobody® according to any of aspects H-1 to H-71, a polypeptide according to any of aspects K-1 to K-19, a compound or construct according to any of aspects L-1 to L-81, or a monovalent construct according to any of aspects L-82 to L-85 in the preparation of a pharmaceutical composition for prevention and/or treatment of at least one Dkk-1 -associated diseases or disorders; and/or for use in one or more of the methods according to aspects R-1 to R-4.

Aspect R-6: Amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, Nanobody® according to any of aspects H-1 to H-71, polypeptide according to any of aspects K-1 to K-19, compound or construct according to any of aspects L-1 to L-81, monovalent construct according to any of aspects L-82 to L-85; or composition according to aspect O-2 or O-3 for the prevention and/or treatment of at least one Dkk-1 -associated diseases or disorders.

Aspect S-1: Part or fragment of an amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, or of a Nanobody® according to any of aspects H-1 to H-71.

Aspect S-2: Part or fragment according to aspect S-1, that can specifically bind to Dkk-1.

Aspect S-3: Part of fragment according to aspect S-2, that can specifically bind Dkk-1 but not Dkk-2, Dkk-3 or Dkk-4.

Aspect S-4: Part of fragment according to aspect S-2, that can specifically bind Dkk-1, Dkk-2 and Dkk-4 but not Dkk-3; or that can specifically bind Dkk-1 but not Dkk-2, Dkk-3 or Dkk-4.

Aspect S-5: Part of fragment according to any of aspects S-1 to S-4, that inhibits and/or blocks binding of LRP5/6 to Dkk-1.

Aspect S-6: Part of fragment according to any of aspects S-1 to S-5, that competes with LRP5/6 for binding to Dkk-1.

Aspect S-7: Part of fragment according to any of aspects S-1 to S-6, that binds to the LRP5/6 binding site on Dkk-1.

Aspect S-8: Part of fragment according to any of aspects S-1 to S-7, that binds to at least one of amino acid residues W212, R242, K217, R209, H210, L266, or H267 on Dkk-1.

Aspect S-9: Part of fragment according to any of aspects S-1 to S-8, that binds to at least one of amino acid residues R242 or H210 on Dkk-1.

Aspect S-10: Part of fragment according to any of aspects S-1 to S-9, that inhibits and/or blocks binding of BHQ880 to Dkk-1.

Aspect S-11: Part of fragment according to any of aspects S-1 to S-10, that competes with BHQ880 for binding to Dkk-1.

Aspect S-12: Part of fragment according to any of aspects S-1 to S-11, that binds to the BHQ880 binding site on Dkk-1.

Aspect S-13: Part of fragment according to any of aspects S-1 to S-12, that inhibits and/or blocks binding of RH2-18 to Dkk-1.

Aspect S-14: Part of fragment according to any of aspects S-1 to S-13, that competes with RH2-18 for binding to Dkk-1.

Aspect S-15: Part of fragment according to any of aspects S-1 to S-14, that binds to the RH2-18 binding site on Dkk-1.

Aspect S-16: Part of fragment according to any of aspects S-1 to S-15, that inhibits and/or blocks binding of 11H10 to Dkk-1.

Aspect S-17: Part of fragment according to any of aspects A S-1 to S-16, that competes with 11H10 for binding to Dkk-1.

Aspect S-18: Part of fragment according to any of aspects S-1 to S-17, that binds to the 11H10 binding site on Dkk-1.

Aspect S-19: Part of fragment according to any of aspects S-1 to S-18, that inhibits and/or blocks binding of Kremen to Dkk-1.

Aspect S-20: Part of fragment according to any of aspects S-1 to S-19, that competes with Kremen for binding to Dkk-1.

Aspect S-21: Part of fragment according to any of aspects S-1 to S-20, that binds to the Kremen binding site on Dkk-1.

Aspect S-22: Part of fragment according to any of aspects S-1 to S-21, that binds to at least one of amino acid residues R197, R209, K214, or K232 on Dkk-1.

Aspect S-23: Part of fragment according to any of aspects S-1 to S-22, that binds to at least one of amino acid residues R197 or K232 on Dkk-1.

Aspect S-24: Part of fragment according to any of aspects S-1 to S-23, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway.

Aspect S-25: Part of fragment according to aspects S-24, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than BHQ880.

Aspect S-26: Part of fragment according to aspects S-24, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than RH2-18.

Aspect S-27: Part of fragment according to aspects S-24, that neutralizes the inhibitory effect of Dkk-1 on the Wnt/beta-catenin signaling pathway equally or better than 11H10.

Aspect S-28: Part of fragment according to any of aspects S-1 to S-27, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction.

Aspect S-29: Part of fragment according to aspects S-28, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than BHQ880.

Aspect S-30: Part of fragment according to aspects S-28, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than RH2-18.

Aspect S-31: Part of fragment according to aspects S-28, that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction equally or better than 11H10.

Aspect S-32: Part of fragment according to any of aspects S-1 to S-31, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation.

Aspect S-33: Part of fragment according to aspects S-33, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than BHQ880.

Aspect S-34: Part of fragment according to aspects S-33, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than RH2-18.

Aspect S-35: Part of fragment according to aspects S-33, that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation equally or better than 11H10.

Aspect S-36: Part of fragment according to any of aspects S-1 to S-35, that increases bone mass in a subject.

Aspect S-37: Part of fragment according to aspect S-36, that increases bone mass in a subject equally or better than BHQ880.

Aspect S-38: Part of fragment according to aspect S-36, that increases bone mass in a subject equally or better than RH2-18.

Aspect S-39: Part of fragment according to aspect S-36, that increases bone mass in a subject equally or better than 11H10.

Aspect S-40: Part of fragment according to any of aspects S-1 to S-39, that reduces bone lesions.

Aspect S-41: Part of fragment according to aspect S-40, that reduces bone lesions equally or better than BHQ880.

Aspect S-42: Part of fragment according to aspect S-40, that reduces bone lesions equally or better than RH2-18.

Aspect S-43: Part of fragment according to aspect S-40, that reduces bone lesions equally or better than 11H10.

Aspect S-44: Part of fragment according to any of aspects S-1 to S-43, that reduces tumor burden.

Aspect S-45: Part of fragment according to aspect S-44, that reduces tumor burden equally or better than BHQ880.

Aspect S-46: Part of fragment according to aspect S-44, that reduces tumor burden equally or better than RH2-18.

Aspect S-47: Part of fragment according to aspect S-44, that reduces tumor burden equally or better than 11H10.

Aspect S-48: Part of fragment according to any of aspects S-1 to S-47, that modulates the inhibitory effect of Dkk-1 on Wnt signaling.

Aspect S-49: Part of fragment according to aspect S-48, wherein said amino acid sequence modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as BHQ880.

Aspect S-50: Part of fragment according to aspect S-48, wherein said amino acid sequence modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as RH2-18.

Aspect S-51: Part of fragment according to aspect S-48, wherein said amino acid sequence modulates the inhibitory effect of Dkk-1 on Wnt signaling via the same mechanism of action as 11H10.

Aspect S-52: Part of fragment according to any of aspects S-1 to S-51, that can specifically bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/litre or less, and preferably 10^{-7} to 10^{-12} moles/litre or less and more preferably 10^{-8} to 10^{-12} moles/litre.

Aspect S-53: Part or fragment according to any of aspects S-1 to S-52, that can specifically bind to Dkk-1 with a rate of association (k_{on}-rate) of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$.

Aspect S-54: Part or fragment according to any of aspects S-1 to S-53, that can specifically bind to Dkk-1 with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Aspect S-55: Compound or construct, that comprises or essentially consists of one or more parts or fragments according to any of aspects S-1 to S-54, and optionally further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers.

Aspect S-56: Compound or construct according to aspect S-55, in which said one or more other groups, residues, moieties or binding units are amino acid sequences.

Aspect S-57: Compound or construct according to aspect S-55 or S-56, in which said one or more linkers, if present, are one or more amino acid sequences.

Aspect S-58: Nucleic acid or nucleotide sequence, that encodes a part or fragment according to any of aspects S-1 to S-54 or a compound or construct according to aspect S-57.

Aspect S-59: Composition, comprising at least one part or fragment according to any of aspects S-1 to S-54, compound or construct according to any of aspects S-55 to S-57, or nucleic acid or nucleotide sequence according to aspect S-58.

Aspect T-1: Derivative of an amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1, or of a Nanobody® according to any of aspects H-1 to H-71.

Aspect T-2: Derivative according to aspect T-1, that can specifically bind to Dkk-1.

Aspect T-3: Derivative according to any of aspects T-1 or T-2, that can specifically bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/litre or less, and preferably 10^{-7} to 10^{-12} moles/litre or less and more preferably 10^{-8} to 10^{-12} moles/litre.

Aspect T-4: Derivative according to any of aspects T-1 to T-3, that can specifically bind to Dkk-1 with a rate of association (k_{on}-rate) of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$.

Aspect T-5: Derivative according to any of aspects T-1 to T-4, that can specifically bind to Dkk-1 with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Aspect T-6: Derivative of a polypeptide according to any of aspects K-1 to K-19 or compound or construct according to any of aspects L-1 to L-85.

Aspect T-7: Derivative according to aspect T-6, that can specifically bind to Dkk-1.

Aspect T-8: Derivative according to any of aspects T-6 or T-7, that can specifically bind to Dkk-1 with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter.

Aspect T-9: Derivative according to any of aspects T-6 to T-8, that can specifically bind to Dkk-1 with a rate of association (k_{on}-rate) of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$.

Aspect T-10: Derivative according to any of aspects T-6 to T-9, that can specifically bind to Dkk-1 with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Aspect T-11: Derivative according to any of aspects T-1 to T-10, that has a serum half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 per se, Nanobody® according to any of aspects H-1 to H-71 per se, polypeptide according to any of aspects K-1 to K-19 or compound or construct according to any of aspects L-1 to L-85 per se.

Aspect T-12: Derivative according to any of aspects T-1 to T-11, that has a serum half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably

more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence according to any of aspects A-1 to A-71, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, F-1 to F-25 or G-1 per se, Nanobody® according to any of aspects H-1 to H-71 per se, polypeptide according to any of aspects K-1 to K-19 or compound or construct according to any of aspects L-1 to L-85 per se, respectively.

Aspect T-13: Derivative according to any of aspects T-1 to T-12, that has a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more; for example, at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

Aspect T-14: Derivative according to any of aspects T-1 to T-13, that is a pegylated derivative.

Aspect T-15: Compound or construct, that comprises or essentially consists of one or more derivatives according to any of aspects T-1 to T-14, and optionally further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers.

Aspect T-16: Compound or construct according to aspect T-15, in which said one or more other groups, residues, moieties or binding units are amino acid sequences.

Aspect T-17: Compound or construct according to aspect T-16, in which said one or more linkers, if present, are one or more amino acid sequences.

Aspect T-18: Nucleic acid encoding a compound or construct according to aspect T-16 or T-17.

Aspect T-19: Composition, comprising at least one derivative to any of aspects T-1 to T-14, compound or construct according to any of aspects T-15 to T-17, or nucleic acid or nucleotide sequence according to aspect T-18.

FIGURE LEGENDS

Figure 1A and B: Llama serum titrations as described in Example 3. A: Serum titrations against human DKK-1 (Day 28); B: Serum titrations against human and mouse Dkk-1 (llama 075).

Figure 2: Characterization of monovalent wild-type Nanobodies® for blocking the interaction of human Dkk-1 with the LRP5 receptor (FACS competition assay) as described in Example 8.1.

Figure 3A and B: Dose response plots for effects of wild-type Nanobodies® on human Dkk-1 antagonism of Wnt-3a signaling in luciferase reporter assays (KS483 reporter cell line) as described in Example 8.2.

Figure 3C: Single dose effect of wild-type monovalent Nanobodies® on mouse Dkk-1 antagonism of Wnt-3a signaling in luciferase reporter assay (KS483 reporter cell line) as described in Example 8.2.

Figure 4A-C: Dose response plots for effects of wild-type monovalent Nanobodies® on Dkk-1 antagonism of BMP2-stimulated ALP assay (and on basal ALP activity) in KS483 cell line as described in Example 8.3.

Figure 5: Characterization of humanized bispecific Nanobodies® (vs. wild-type monovalent and bispecific controls) for blocking the interaction of human Dkk-1 with the LRP5 receptor (FACS competition assay) as described in Example 12.1.

Figure 6: Dose response plots for effects of humanized, bispecific Nanobodies® vs wild-type monovalent Nanobodies® on DKK-1-antagonized Wnt 3a signaling in KS483 reporter cell line.

Figure 7: Dose dependent inhibition of humanized bispecific Nanobodies on Dkk-1 antagonism of BMP2-stimulated ALP assay (and on basal ALP activity) in KS483 cell line as described in Example 13.

Figure 8: Inhibition by the humanized bispecific Nanobodies of the antagonistic effect of Dkk-1 on basal mineralization of KS483 cells.

Figure 9: Effect of humanized bispecific Dkk-1 Nanobodies on basal and BMP2-stimulated mineralization of KS483 cells in the absence or presence of Dkk-1.

EXAMPLES

Example 1: Materials

Human Dkk-1 was obtained from R&D Systems, Inc. (Minneapolis, MN) as a carrier-free recombinant protein (fused with a C terminal 10X His tag) produced in *Sf* 21 cells using a baculovirus expression system.

Mouse Dkk-1 was obtained from R&D Systems, Inc. (Minneapolis, MN) as a carrier-free recombinant protein (fused with a C terminal 10X His tag) produced in a mouse myeloma cell line, NS0.

Biotinylated human and mouse Dkk-1 were generated in-house using standard protocols (using biotinamido hexanoic acid 3-sulfo N-hydroxy succinimide ester [Sigma, B1022-10MG, 024K5001]) and a 15-molar excess of biotin reagent vs human dkk-1 (reaction was incubated for 1.5 hrs at room temperature).

Biotinylated polyclonal anti-human Dkk-1 antibody was obtained from R&D Systems Inc. (Minneapolis, MN).

Llama IgG (h&l) antibody HRP (horse radish peroxidase) conjugated is a polyclonal antibody against llama IgG raised in goat obtained from Bethyl Labs (Montgomery, TX).

A bulk (non clonal) stable HEK293LRP5 cell line was obtained from Proctor and Gamble Pharmaceuticals (P&GP).

Example 2: Immunizations

Two llamas (073 and 074) were immunized with human Dkk-1 and one llama (075) was immunized with mouse Dkk-1 according to the scheme outlined in Table C-1.

After completion of the protocol, immune responses were analyzed by ELISA: serial dilutions (starting dilution: 1/500) of serum samples collected at days 0, 28, 39, 43, 46, 50 were incubated with human or mouse DKK-1 (2µg/ml in DPBS) adsorbed to a 96 well ELISA plate. Bound llama IgG was detected by goat anti-Llama IgG (Bethyl A160-100; Montgomery, TX). Results at day 28 are shown in Figures 1A and B.

Example 3: Library construction

RNA extracted from peripheral blood lymphocytes (PBL) and lymph node (LN) obtained from llama was used as starting material for RT-PCR to amplify Nanobody® encoding gene fragments. These fragments were cloned into a phagemid vector. Phage was prepared according to standard methods and stored after filter sterilization at 4ºC for further use. The characteristics of the constructed libraries are shown in Table C-2.

Example 4: Selections

Selections were carried out with the above libraries using various conditions as summarized in Table C-3.

Two rounds of selection were performed with reducing concentrations of biotinylated human DKK-1 (in solution, followed by capture on streptavidin beads, followed by washing and trypsin elution), or by alternating between human and mouse DKK-1. Each selection output was analyzed for enrichment factor (# phage present in eluate relative to control) and plated for further analyses. Colonies were picked, grown in 96-well plates and stored in 20% glycerol in -80C. Copies of the output plates were grown in 96 deep well plates (1 ml volume) and induced by adding IPTG for Nanobody® expression. Periplasmic extracts (volume: ~ 90 µl) were prepared according to standard methods (see for example the prior art and applications filed by Ablynx N.V. cited herein).

Example 5: Primary screening (binding to human and mouse Dkk-1 in ELISA)

Periplasmic extracts from populations resulting from a range of different selection conditions were analyzed for their ability to bind human Dkk-1 and for binding to mouse Dkk-1 in parallel (ELISA; with biotinylated human or mouse Dkk-1 adsorbed to streptavidin plate).

Example 6: Sequence analysis

357 clones specific for human Dkk-1, many of which were shown to be cross-reactive with mouse Dkk-1 were subjected to sequence analysis (AGOWA, Middlesex, UK), resulting in the identification of a set of 291 different, unique Nanobody® sequences.

Based on the amino acid sequence homology (especially homology for CDR3) all clones were classified into 24 different families or single sequence representatives. Members of the same family are believed to have similar behavior in the different assays described in this patent application, though small differences in functional properties are possible. One or several representatives per family were chosen for further characterization.

Example 7: Nanobody® expression and purification in *E. coli*

Selected Nanobodies® were expressed in *E.coli* as c-myc, His6-tagged proteins in a culture volume of 50 mL. Expression was induced by addition of 1 mM IPTG and allowed to continue for 4h at 37ºC. After spinning the cell cultures, periplasmic extracts were prepared by freeze-thawing the pellets. These extracts were used as starting material for immobilized metal affinity chromatography (IMAC). Nanobodies® were eluted from the column with 150 mM imidazole and subsequently buffer-exchanged to PBS.

For production of the wild-type monovalent DKK-1 Nanobodies® in *E. coli*, recombinant *Escherichia coli* strains TG1 containing WT anti-DKK1 Nanobody® constructs were inoculated in 25mL (for shake flask) of 250ml (for bioreactor) LB medium + Kan (50mg/L) + 2% glucose and incubated ON at 37ºC and 200 rpm. For the 21C12, 18B5, 10A5 constructs 1% of the preculture was transferred into a 250ml shaker flask containing 200ml TB medium supplemented with the appropriate antibiotics and further grown at 37°C until OD600~1.5-2. The cultures were then induced with 1mM IPTG and further grown at 28ºC over night. For some constructs a Biostat B plus 10L containing TB medium + Kan (50mg/L) was inoculated with 1/50 of the obtained overnight pre-culture and further grown at 37°C during the following batch phase to obtain biomass. After 3 hours, the cultures were induced with 1mM IPTG and further grown at 30ºC for another 3 hours during induction phase until OD600>10.

The cultures were harvested by centrifugation Sigma 8K10 rotor ; 7000rpm; 20'; 4°C), after which the clarified fermentation broth was stored at 4°C and the cell pellets were stored at -20°C. Localization study demonstrated that the protein was secreted both to the periplasm and the medium.

For purification, periplasmic extracts were prepared by re-suspending the pellets in peri-buffer (50mM NaH2PO4, 300mM NaCl pH 8.0) and incubating for 40 minutes at 4°C on a shaking platform at 100 rpm. The suspensions were centrifuged at 7000 rpm for 40 minutes to clear the cell debris from the periplasmic extract, followed by a filtration step using a 0.22 µm filter. For purification of Nanobodies® expressed in the medium, the medium was first treated with 100mg/L reduced gluthation, followed by a filtration step using a 0.22µm filter.

The HIS6-tagged anti-DKK1 Nanobodies® were then captured and purified by immobilized metal affinity chromatography (IMAC, NiSFF, GE Healthcare) followed by a concentration step on a smaller IMAC column. Finally, a gel filtration chromatography was performed on a Superdex 75 column (10/300GL, GE Healthcare) in D-PBS The OD280 nm was measured and the concentrations for the different Nanobodies® were calculated. Samples were after sterile filtration stored at -20C.

The Nanobody® was captured via affinity chromatography on MabCap A (POROS). For this, the sample was loaded in PBS and eluted with a step gradient of 100mM glycine pH2.6.

The protein was further polished via AEX on POROS 50HQ followed by size exclusion on Superdex75 (GE Healthcare). For the polishing step, the sample was loaded in 25mM Tris pH 8.8 (buffer A) and eluted with a linear gradient of 10CV from buffer A to 100% Buffer B (25mM Tris pH 8.8+ 500mM NaCl). The sample was concentrated using Centricon (Sartorius Vivaspin 5000Da MWCO) and 50mM OGP (Glycon) was added to remove LPS and incubated over night @4°C. For the SEC polish step by size exclusion on Superdex75, the sample was loaded in D-PBS on a Superdex75 XK26/60 column (GE Healthcare,).

The OD280 nm was measured and the concentrations for the different Nanobodies® were calculated. Samples were after sterile filtration stored at -20°C. Additional purification steps were included for Nanobody® samples to be tested in cell-based assays (as shown in examples 8 below). Sequences of a panel of purified Nanobodies® are depicted in Table A-1.

Example 8: Characterization of monovalent Nanobodies® *in vitro*

8.1 Blocking the interaction between human Dkk-1 and human LRP5 receptor

Nanobodies® representing each sequenced family were purified (Example 7) and screened to identify those which block the interaction between human Dkk-1 and human LRP5 receptor in a FACS-based competition assay. The Nanobodies® were pre-incubated with human Dkk-1 prior to incubation with the HEK293:hLRP5R cell line, followed by detection of Dkk-1 with biotinylated polyclonal anti-human Dkk-1 antibody (R&D Systems) and streptavidin-PE as follows.

A bulk stable (non clonal) stable HEK293LRP5 cell line was grown up to 8 x 10^6 cells (in MEM alpha (Invitrogen, Cat 32571-028) + 10% FBS (invitrogen, Cat 10270-106) + 1% Pen/Step (Invitrogen, Cat 15140-122) + 1% NEAA (Non Essential Amino Acids, Invitrogen, Cat 1140-035) + 100µg/ml Zeocin (Invitrogen, Cat 45-0430). Cells were counted using Tryptan Blue (Invitrogen, Cat 15250-061) and haematocytometer.

For the FACS array, a 1/2 serial dilution of the Nanobodies® (starting at 500nM) was incubated with 5nM hDKK1 (final concentration) (R&D systems, Cat 1096-DK/CF) for 1 hour at 4°C. The cells were brought at a concentration of 2x10^6 cells/ml in FACS buffer (PBS 1x Invitrogen: 141190-0940)) and 100µl was transferred (= 2x10^5 cells) in a V-bottom 96-well plate. They were centrifuged at 250g for 5 min at 4ºC, resuspended in 100µl hDKK1/Nanobody® mix and incubated for 30 min at 4ºC. Then, the mix was washed 3 times with FACS buffer, the cells were resuspended in 100µl diluted goat anti-hDKK1-biotin (0,100 mg/ml stock, R&D systems; Cat BAF1096; final concentration 5ug/ml) and incubate for 30 min at 4ºC. The mix was again washed 3 times with FACS buffer. Then, appropriate cells were resuspended in 100µl diluted Streptavidin-PE (BD Pharmingen, Cat 554061; final dilution: 1/1000) and incubated for 30 min, at 4°C in the dark. The mix was washed 3x with FACS buffer and cells were resuspended in 100µl FACS buffer + 5nM TOPRO3 (Molecular Probes T3605 Lot 18C1-2).

First a P1 population which represents more than 80% of the total cell population was selected. In this gate 20000 cells were counted. From this population the TOPRO3+ cells (dead cells) were excluded. For this P1/TOPRO3- population the mean PE value is calculated

Five Nanobodies® from 4 different families were identified, which were able to block the LRP5/Dkk-1 interaction with varying efficacy and potencies (Figure 2 and Table C-4). Two representatives of family were shown to antagonize human Dkk-1 binding to LRP5 and a further six

Nanobodies® representing sequence variants of this family were identified in the sequence analysis of all binding populations.

An overview of functional (LRP5 blocking) Nanobodies® and the selection conditions that were used to generate them is given in Table C-6 (Note: 1B9 is a high affinity non-blocking Nanobody®).

8.2 Ability of monovalent wild type Nanobodies® to inhibit Dkk-1 antagonism of Wnt luciferase reporter assays *in vitro*

Nanobodies® were also tested for their ability to inhibit human (and mouse) Dkk-1-mediated antagonism of Wnt3a induced signalling in both the mouse KS483 and C3H10T1/2 Wnt reporter cell lines *in vitro* (WO 06/015373, WO 07/084344; WO 08/097510; van Bezooijen RL et al. 2007, J. Bone Miner. Res. 22: 19-28). Dose response curves for the panel of Nanobodies® (highly purified samples depicted as (p) are shown in Figures 3A and B for human Dkk-1 and in Figure 3C for mouse Dkk-1.

8.3 Ability of monovalent wild type Nanobodies® to inhibit basal or Dkk-1-antagonised BMP2-stimulated alkaline phosphatase activity (ALP) *in vitro*

The purified wild-type Nanobodies® were tested for the ability to inhibit basal, BMP2-stimulated and Dkk-1 antagonized BMP-2-stimulated Alkaline Phosphatase activity in the mouse KS483 cell line (Van der Horst Get al. 2002, Bone 31: 661–669; van der Horst et al. 2005, J Bone Mineral Res. 20:1867).

Dose response curves showing the effect of Nanobodies® on basal ALP and on Dkk-1 antagonised BMP2-stimulated ALP are shown in Figures 4A-C.

Example 9: Humanization of Nanobodies® 4E4 and 16A12

Humanized variants of each Nanobody® (8 variants of each) were generated using typical molecular engineering approaches. The variants were produced in Pichia (example 11) and were tested for binding to human dkk-1 (data not shown). One final variant of each (4E4hum 7 and 16A12hum7) were chosen for formatting and further characterization.

Sequences of the humanized Nanobody® variants are summarized in Table A-2.

Example 10: Construction of bispecific humanized Nanobodies® via fusion with anti-HSA Nanobody®

Nanobody® constructs were generated with the final humanized (hum 7) variant of each anti-Dkk-1 Nanobody® (4E4 and 16A12). The humanized anti-Dkk-1 Nanobody® was fused to a humanized

Nanobody® specific for HSA (Alb-8) at the C terminus, via a flexible 9GS linker (GGGGSGGGS; SEQ ID NO: 1823). Sequences of the final humanized, bispecific Nanobodies® are shown in Table A-3.

Example 11: Production of humanized, bispecific Nanobodies® in *Pichia pastoris*

The constructs for the humanized bispecific Nanobodies® were cloned into pPICZalphaA (Invitrogen, Carlsbad, CA) and transformed in *Pichia pastoris*. Fermentation of the anti-Dkk1 Nanobodies® in the *P. pastoris* X-33 strain was performed as described below. The fusion to the alpha-factor signal peptide directs the expressed Nanobodies® into the culture supernatant.

In a 1 L baffled erlenmeyer, 250 mL of the standard YPD medium with 2% glucose was inoculated with a 0.250 mL aliquot from a glycerol stent (RCB stent) and incubated at 30°C on an incubator shaker at 200 rpm for 20 à 24 h. The fermenters were inoculated with this pre-culture to obtain an initial OD of 0.2 in the fermenter. Fermentations were performed in table-top glass bioreactors of Sartorius Stedim Biotech (10 or 5 liter working volume, Biostat B plus reactors). The reactors were equipped with electrodes to monitor pH, temperature and dissolved oxygen. The processes were controlled and registered via the MFCS-win 3.0 software of Sartorius Stedim Biotech.

Two different fermentation strategies were performed to produce the anti-Dkk Nanobodies®. Primarily, a fermentation protocol including a medium switch was performed. This fermentation strategy started with a biomass production phase (24 hrs) in 10L of BGCM medium (in a 10L reactor); followed by a medium switch to 5L of the BMCM medium (with 1% of methanol) to initiate Nanobody® production. MeOH was added during the fermentation each time the dissolved oxygen spiked. Each 12 hrs, an extra amount (1L) of the complex BMCM medium was added to the culture. The temperature was kept at 30°C and the dissolved oxygen was controlled at 30% (via stirrer speed and pure oxygen additions). The *Pichia* cells were separated from the culture medium via centrifugation (Sigma 8K10 rotor; 7000rpm; 20'; 4°C) and the medium (7 L) was 0.2µm filtered.

The second fermentation protocol used for anti-Dkk-1 Nanobody® production was a fedbatch fermentation strategy. This fermentation started with a batch fermentation in 2,5L medium (in a 5L reactor). When the carbon source (glycerol) was totally consumed, further biomass was build-up by feeding a glycerol-yeast extract feed solution (60% (w/v) glycerol with 20% (w/v) yeast extract) to the cell culture. When a wet cell weight of approximately 400 g/L was reached, the induction with 100% MeOH was started. The induction phase started with an adaptation phase (a feed of 1.5 mL/h.L initial fermentation volume for 2 hours, followed by a feed of 3 mL/h.L initial fermentation volume for the next 2 hours). After the adaptation phase, a constant MeOH feed of 4 mL/h.L initial fermentation volume was performed. The temperature was kept at 30°C and the dissolved oxygen was controlled at 30% (via stirrer speed and pure oxygen additions). After an induction time of 24 hrs the *Pichia* cells

were separated from the culture medium via centrifugation (Sigma 8K10 rotor; 7000rpm; 20'; 4°C) and the medium (3,5 L) was 0.2µm filtered.

The Nanobody® was captured via affinity chromatography on MabCap A (POROS). For this, the sample was loaded in PBS and eluted with a step gradient of 100mM glycine pH2.6.

The protein was further polished via AEX on POROS 50HQ followed by size exclusion on Superdex75 (GE Healthcare). For the polishing step, the sample was loaded in 25mM Tris pH 8.8 (buffer A) and eluted with a lineair gradient of 10CV from buffer A to 100% Buffer B (25mM Tris pH 8.8+ 500mM NaCl). The sample was concentrated using Centricon (Sartorius Vivaspin 5000Da MWCO) and 50mM OGP (Glycon) was added to remove LPS and incubated over night @4°C. For the SEC polish step by size exclusion on Superdex75, the sample was loaded in D-PBS on a Superdex75 XK26/60 column (GE Healthcare,).

The OD280 nm was measured and the concentrations for the different Nanobodies® were calculated. Samples were after steriie filtration stored at -20°C.

Example 12: Characterization of humanized, bispecific anti-Dkk-1 Nanobodies®

12.1 FACS-based competition assay for binding to hLRP5

A FACS-based competition assay for binding to hLRP5 was performed as described in Example 8.1.

Humanized, bispecific Nanobodies® were compared alongside the wild-type parental molecules both in monovalent and in the same bispecific (Alb-8 fusion) format. Inhibition curves and IC50 values are shown in Figure 5 and Table C-5.

12.2 Wnt luciferase assay

A Wnt luciferase assay was performed as described in Example 8.2. Dose response curves are shown in Figure 6.

Example 13: Validation of humanized nanobodies to Dkk1 on their ability to inhibit the effect of Dkk1 on basal and stimulated bone formation in vitro using an osteogenic cell line

13.1 Effect of two humanized Dkk1 Nanobodies on basal and BMP2-stimulated ALP activity in the absence or presence of human Dkk1

Mouse osteogenic KS483 cells were seeded in a 96 wells-plate and treated at confluence, day 3 or 4, with recombinant human BMP2 (100 ng/ml), recombinant human Dkk1 (1000 ng/ml), and/or dose range of the humanized bispecific Dkk1 Nanobodies4E4 and 16A12. ALP activity was kinetically measured in the cell layer after 4 days stimulation (van Bezooijen et al., JBMR 2007, 22: 19).

Humanized bispecific Dkk1 Nanobodies 4E4 and 16A12 dose-dependently inhibited human Dkk1 antagonized ALP activity, either under basal or BMP-stimulated conditions (Figure 7). The humanized Nanobodies 4E4 and 16A12 were equally potent. The two humanized Nanobodies had no effect on basal or BMP-stimulated ALP activity, i.e. in the absence of recombinant human Dkk1.

13.2 Effect of two humanized bispecific Dkk1 Nanobodies on basal and BMP-stimulated mineralization in the absence or presence of human Dkk1

Mouse osteogenic KS483 cells were seeded in a 24 wells-plate. After cells were confluent, day 3 or 4, they were cultured under osteogenic conditions (i.e. from confluency onwards 50 µg/ml ascorbic acid and from bone nodule formation onwards (day 9-11) ascorbic acid and 5 mM beta-glycerophosphate). Cells were treated with Dkk1, BMP2 and/or the Nanobodies 4E4 and 16A12 for 4 days as indicated. From then onwards, cells were cultured only without or with the Nanobodies until 18 days of culture. Mineralization was analyzed using Alizarin Red staining and quantified by dissolving the Alizarin Red from the mineralized nodules (Van Bezooijen et al., J. Exp. Med. 2004, 199: 805).

The two humanized bispecific Dkk-1 Nanobodies 4E4 and 16A12 tended to block the inhibitory effect of Dkk-1 on basal mineralization of KS483 cells. They significantly inhibited the antagonistic effect of Dkk-1 on BMP2-stimulated mineralization (Figures 8 and 9). The Nanobodies had no effect on basal or BMP2-stimulated bone formation.

TABLES

Table A-1: Amino acid sequences of Nanobodies® that bind DKK-1

Nanobody®	SEQ ID NO:	Sequences
PDKKPMP4E4	1589	EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQTPGKEREGVS CISSSDGSTYYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAA EVPSSCGGYSGSYYYTDDELDFWGQGTQVTVSS
PDKKPMP16A12	1590	EVQLVESGGGLVQAGGSLRLSCAASGRTFNNYAMGWFRQTPGKEREFVA AISWIIGSTYYADSVKGRFTISRDNAKNTVYLQMNGLKPEDTAVYYCAA EGGYSGTYYYTGDFDSWGQGTQVTVSS
PDKKPMP1A4	1591	EVQLVESGGGSVQAGGSLRLSCAVSGRTFSSYSMGWFRQAPGKEREFVA AISWSGGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPADTAVYYCAT DESRLYGGKRPKMLYDYWGQGTQVTVSS
PDKKPMP1A8	1592	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVA AISWSGGSTVYTDSVKGRFTISKDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGRYYGHSRARYDYWGQGTQVTVSS
PDKKPMP1A10	1593	EVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGSYYGHSRARYDYWGQGTQVTVSS
PDKKPMP1B3	1594	EVQLVKSGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVS AISWSGGSTVYADSVKGRFTISRDNAENTVYLQMNSLKPEDTAVYYCAA GNGGSRYGHSRARYDYWGQGTQVTVSS
PDKKPMP1B5	1595	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGRTYGHSRARYDYWGQGTQVTVSS
PDKKPMP1B9	1596	EVQLVKSGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVA AINWSGGSTIYADSVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCAA GNGGRSYGHSRARYDYWGQGTQVTVSS
PDKKPMP5A1	1597	EVQLVESGGGLVQPGGSLRLSCAASGRTFSNNAVGWFRQAPGKERQFVA AISWTAGTTYYADSVKGRFTISRDNATNTVYLQLNSLKPEDTAVYFCAA GAGRDRGFSRAQYAYWGQGTQVTVSS
PDKKPMP5A2	1598	EVQLVESGGGLVQAGGSLRLSCAASGRTLSRSAMGWFRQAPGKEREFVA AINWSGGSTYYGDFVKGRFTISRDSAKNTVYLQMNSLKPEDTAVYYCAA GNGGSYYGHSRARYDYWGQGTQVTVSS
PDKKPMP5A8	1599	EVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKERDFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGSYYGHSRARYDYWGQGTQVTVSS
PDKKPMP5A12	1600	EVQLVESGGGLVQAGGSLRLSCAASGRTFSNFAMGWFRQAPGKEREFVA AINWSGASTVYADSVKGRFTISKDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGSYYGHSRARYDYWGQGTQVTVSS
PDKKPMP5B3	1601	EVQLVESGGGLVQAGGSLRLSCEASGRTFSNYATGWFRQAPGKEREFVA AISWSGGSTLYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGRNYGHSRARYDYWGQGTQVTVSS
PDKKPMP5D11	1602	KVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVA AISWSGASAIYADSVKGRFTISRDNAKNTVYLQMNSLNAEDTAVYYCAA GNGGSYYGHSRARYDYWGQGTQVTVSS
PDKKPMP5E9	1603	EVQLVESGGGLVQAGGSLRLSCVVSGGTFNVYAMGWFRQAPGRLREIVA AISRTGSSIYYADSVKGRFTISRDDAKKTVYLQMNSLKSEDTAVYYCAV DGRANGERPAVAGYNYWGQGTQVTVSS
PDKKPMP5G4	1604	EVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVG AINWSGGSTIYADSVKGRFTISRDNAENTVYLQMNSLRPEDTAVYYCAA GNGGRNYGHSRARYEYWGQGIQVTVSS
PDKKPMP10A5	1605	EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKEREGVS CLSSSDGSTYYADSVKGRFTISTDNAKNTVYLQMNSLKPEDTAVYYCAA VEPAHCAYQSGVTAYEIYGMDYWGKGTLVTVSS
PDKKPMP10A6	1606	EVQLVESGGGLVQAGGSLRLSCVVSGGTFNVYAMGWFRQAPGKLREIVA AISRTGSSIYYADSVKGRFTISRDDAKKTVYLQMNSLKSEDTAVYYCAV DGRANGERPAVAGYNYWGQGTQVTVSS

PDKKPMP10A11	1607	EVQLVESGGGLVQAGGSLRLSCVVSGGTFSVYAMGWFRQAPGKLREIVA AISRTGSSIYYTDSVKGRFTISRDDAKKTVYLQMNSLKSEDTAVYYCAV DGRANGERPAVAGYNYWGQGTQVTVSS
PDKKPMP10B1	1608	EVQLVKSGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVA AINWSGSSTVYADSVKGRFTISRDNAENTVYLQMNSLKPEDTAVYYCAA GNGGRNYGHSRARYDYWGQGTQVTVSS
PDKKPMP10B4	1609	EVQLVESGGGLVQAGASLRLSCAASGGTFSRYPMGWFRQAPGKEREFVA AISWNGGSTIYADSVKGRFTISKNNAKNTVYLQMNSLRPEDTAVYYCAA GNGGSYYGHSRARYDYWGQGTQVTVSS
PDKKPMP10C12	1610	EVQLVESGGGLVRAGGSLRLSCVVSGGTFSVYAMAWFRQAPGKLREIVA AISRTGSSIYYTDSVKGRFTISRDDAKKTVYLQMNSLKSEDTAVYYCAI DGRANGERPAVAGYNYWGQGTQVTVSS
PDKKPMP15A9	1611	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVA AISSSASSTVYADSVKGRFAISRDNAKSTVYLQMNSLKPEDTAVYYCAA GNGGRKYGHHRARYDYWGQGTQVTVSS
PDKKPMP15B6	1612	EVQLVESRGGLVQAGGSLRLSCVASGRTFSSYAMGWFRQAPGKEREFVA AISRSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGRLYGHSRARYDYWGQGTQVTVSS
PDKKPMP15F1	1613	EVQLVKSGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVG AINWSGGSTIYADSVKGRFTISRDNAENTVYLQMNSLKPEDSAVYYCAA GNGGRNYGHSRARYEYWGQGIQVTVSS
PDKKPMP16A11	1614	EVQLVESGGGLVQPGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNMVYLQMNSLKPEDTAVYYCAA GNGGRYYNHSRTRYEYWGQGTQVTVSS
PDKKPMP1A3	1615	EVQLVESGGGLVQAGGSLRLSCVVSGGTFSVYAMGWFRQAPGKLREIVA AISRTGSSIYYADSVKGRFTISRDDAKKTVYLQMNSLKSEDTAVYYCAV DGRANGERPAVAGYNYWGQGTQVTVSS
PDKKPMP16B1	1616	EVQLVKSGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVA AISSSGSSTVYADSVKGRFAISRDNAKNTVYLQMNNLKPEDTAVYYCAA GNGGRNYGHSRARYDYWGQGTQVTVSS
PDKKPMP16B3	1617	EVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQASGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNMVYLQMNSLRPEDTAVYYCAA GNGGRTYGHSRARYEYWGQGTQVTVSS
PDKKPMP16B11	1618	EVQLVESGGGLVQSGGSLRLSCVASGSTLDSYDIGWFRQVPGKEREGIS CIRTSEDRTYYSDSVKDRFTISRDNTKNTVYLQMNSLKPEDTAIYYCAT HLPLLASRLIWDSRCTLVEHEYDSWGQGTQVTVSS
PDKKPMP16B12	1619	EVQLVESGGGLVQAGGSLRVSCAASGRTFSSYAMGWFRQAPGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAT DESRLYGGKRPKMLYDYWGQGTQVTVSS
PDKKPMP16A3	1620	EVQLVESGGGLVQAGGSLGLSCAASGRTFSRYAMGWFRQAPGKEREFVA AISWSGGSAVYADSVKGRFTISRDNAENTVYLQMNSLKSEDTALYYCAA GNGGSYYGHSRARYDYWGQGTQVTVSS
PDKKPMP18A4	1621	EVQLVESGGGLVQAGGSLRLSCAASGRAFSNYAMGWFRQAPGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGRQYGHSRARYDYWGQGTQVTVSS
PDKKPMP18A10	1622	EVQLVKSGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVA AISWSGGSTLYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCAA GNGGRTYGHSRARYDYWGQGTQVTVSS
PDKKPMP18A12	1623	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVA AISSSGSSTVYADSVKGRFAISRDNAKNTVYLQMNNLKPEDTAVYYCAA GNGGRNYGHSRARYDYWGQGTQVTVSS
PDKKPMP18B3	1624	EVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQASGKEREFVA AISWTGGSTVYADSVKGRFTISRDNAKNLLYLQMNSLKPEDTAVYYCAA GNGGRTYGHSRARYEYWGQGTQVTVSS
PDKKPMP18B4	1625	EVQLVESGGGLVQAGGSLRLSCAASGRTFSNYPMGWFRQAPEKEREFVA AISWSGGSTAYADSVKGRFTISRDNAENTVYLQMNSLKPEDTAVYYCAA GNGGRNYGHSRARYDYWGQGTQVTVSS
PDKKPMP18B5	1626	EVQLVESGGGLVQAGGSLRLSCAASGFPLDDYAIGWFRQAPGKEREGVS CISSSDGRAVYADSVKGRFTISIDNAKNTVYLQMNSLKPEDTAVYYCAA DPYYSGSYYYTDPDRYDYWGQGTQVTVSS

PDKKPMP18E4	1627	EVQLVESGGGLVQAGGSLRLSCAASGRTISNYAMGWFRQAPGKEREFVA AISWSGGSTIYADSVKGRFTISRDNAKNTVSLQMNSLKPEDTAVYYCAA GNGGSYYGHSRARYDYWGQGTQVTVSS
PDKKPMP18E6	1628	EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKEGEGVS CISSSDGSTYYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAA EYPSSCASGSYYYRDSENYDYWGQGTQVTVSS
PDKKPMP1A1	1629	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPEKEREFVA AISWSGGSTVYADSVKGRFTISRDNAENTVYLQMNSLKPEDTAVYYCAA GNGGRNYGHSRARYDYWGQGTQVTVSS
PDKKPMP1B11	1630	EVQLVESGGGLVRPGGSLRLSCASSASISVFDAMGWYRQAPGKQRERVA EIINDGTTNYAGDVKGRFTISRDNTKNTMYLQMNNLKPEDTAVYYCSAY YTDYDEALEETRGSYDYWGQGTQVTVSS
PDKKPMP5B11	1631	EVQLVESGGGLVQAGGSLRLSCAASGRTFSNAAMGWFRQAPGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGSYSGHSRARYDYWGQGTQVTVSS
PDKKPMP5D3	1632	QVQLVESGGGLVQAGGSLRLSCAVSGRTFSSYTMGWFRQAPGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAT DESRLYGGKRPKMLYDYWGQGTQVTVSS
PDKKPMP16A9	1633	EVQLVESGGGLVQAGGSLRLSCAASGRTFSTYAMGWFRQAPGKERELVA AISWSGGSTYYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAVYYCAA GNGGSKYGHSRARYDYWGQGTQVTVSS
PDKKPMP18A9	1634	EVQLVESGRGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVA AINWSGGSTVYADSVKGRFTISRDNANNTVHLQMNSLKPEDTAVYYCAA GNGGRAYGYSRARYEYWGQGTQVTVSS
PDKKPMP2A1	1635	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWALTRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKWISVRYFSTSSNYDYWGQGTQVTVSS
PDKKPMP2A3	1636	EVQLVESGGGLVQAGGSLGLSCTASGRAFSGSAVGWFRQGPGKEREFVT AINWSGARTYYVDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA ARDRYARVLTDYEYVWWGQGTQVTVSS
PDKKPMP2A7	1637	EVQLVESGGGLVQAGGSLNLSCVLSGRTLWRSNMGWFRQAPGKEREFVG SIDWITTNIYYADSVKGRFTISRDNPKNTFYLQMNSLKPEDTAVYRCAS SGPADARNGERWALWGQGTQVTVSS
PDKKPMP2A10	1638	EVQLVESGGGLVQAGGSLRLSCTASGSAFSGTVVGWFRQAPGKEREFVT AINWSGARTYYADSVKGRFTISRDNAKNTAYLQMNSLRPEDTAVYYCAA GSDRYARVLTEYEYVWWGQGTQVTVSS
PDKKPMP2A11	1639	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWYLSTTYYADSVKGRFTISRDNAKNTAFMQMSSLKAEDTAVYYCAA APRPKRVSVRYFSTSSNYDYWGQGTQVTVSS
PDKKPMP2B9	1640	EVQLVESGGGLVQAGGSLRLSCVLSGRTLWRSNMGWFRQAPGKEREFVG SIDWITTNTYYADSVKGRFTISRDNPKNTFYLQMNSLKPEDTAVYRCAS SGPADARNGERWALWGQGTQVTVSS
PDKKPMP3A5	1641	EVQLVESGGGLVQPGGSLRLSCAASGFPFSIYPMKWVRQAPGKGLEWVS HITRDGGGTMYADSVKGRFTISRDNAKNMLYLQMNSLKPEDTAVYFCAR SAGKVVSRASLENDYRGQGTQVTVSS
PDKKPMP3A9	1642	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKWVSVRYFSTSSNYDYWGQGTQVTVSS
PDKKPMP3A11	1643	EVQLVESGGGLVRAGGSLKLSCTASGRAFSSSAVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAA GRDSHARHLSEYEYMWWGQGTQVTVSS
PDKKPMP3B1	1644	EVQLVKSGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKRISVQYFTTSSNYDYWGQGTQVTVSS
PDKKPMP3B2	1645	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKYVSVRYFTTSSNYDYWGQGTQVTVSS
PDKKPMP3B5	1646	EVQLVESGGGLVQAGGSLRLSCAASGHTFSSYAMGWFRQALGKEREFVA AINWSSRSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GSAYVAGVRRRNAQYDYWGQGTQVTVSS

PDKKPMP3B7	1647	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYANSVEGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPNRVSVRYFSTSSNYDYWGQGTQVTVSS
PDKKPMP3D1	1648	EMQLVESGGGLVQAGGSLRLSCAASGDTFSNYAMGWFRQAPGKEREFVA AINWSSRSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GTAYVAGVRRRKAEYDYWGQGTQVTVSS
PDKKPMP3F6	1649	EVQLVESGGGLVQAGGSLRLSCTASGRAFSSSVVGWFRQAPGKEREIVT AINWSGARTYYADSVKGRFTISRDNAKNTVYLQMNSLKPLDTAVYYCAA GSDRYARVLTEYEYVWWGQGTQVTVSS
PDKKPMP3G3	1650	EVQLVESGGGLVQAGGSLRLSCVLSGRTLSRSNMGWFRQAPGKEREFVA SIDWITINTYYADSVKGRFTISRDNPKNTFYLQMNSLKPEDTAVYRCAS SGPADARNGERWHLWGQGAQVTVSS
PDKKPMP3A1	1651	EVQLVELGGGLVQAGDSLTLSCTASGRTFDNYAMGWFRQAPGKEREFVA AISWSLSRTHYADYVKGRFTISRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKRISVSYFSTTSNYDYWGQGTQVTVSS
PDKKPMP6B2	1652	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVTGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKWVSVRYFSTSSNYDYWGQGTQVTVSS
PDKKPMP6B3	1653	EVQLVESGGGLVQAGDSLTLSCTASGRTFDNYAMGWFRQAPGKEREFVA AISWSLSRTHYADYVKGRFTISRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKRISVSYFSTTSNYDYWGQGTQVTVSS
PDKKPMP6C9	1654	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYGMGWFRQAPGKEREFVA GISWTLGRTYCADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAS DGPDAYNSWGQGTQVTVSS
PDKKPMP6D1	1655	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVG AISWSLSRTHYADSVEGRFIISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKWVSVRYFSTSSNYDYWGQGTQVTVSS
PDKKPMP6D2	1656	EVQLVESGGGLVQPGGSLRLSCAASGFPFSIYPMKWVRQAPGKGLEWVS HITRDGGRTMYADSVKGRFTISRDNAKNMLYLQMNSLKPEDTAVYFCAR SAGKVVSRASLENDYRGQGTQVTVSS
PDKKPMP6E1	1657	EVQLVESGGGLVQAGGSLRLSCAASGDTFSNYAMAWFRQAPGKEREFVA AINWSSRSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GTSYVAGIRRRNAEYLYWGQGTQVTVSS
PDKKPMP6E2	1658	EVQLVESGGGLVQPGGSLRLSCAASGFPFSIWPMKWVRQAPGKGLEWVS HITRDGGRTMYADSVKGRFTISRDNAKNMLYLQMNSLKPEDTAVYFCAR SAGKVVSRASLENDYRGQGTQVTVSS
PDKKPMP6E10	1659	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AVSWSLSRTHYADSVEGRFIISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKWISVRYFTTSSNYDYWGQGTQVTVSS
PDKKPMP6G7	1660	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKRISVQYFTTSSNYDYWGQGTQVTVSS
PDKKPMP13B2	1661	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYGMGWFRQAPGKEREFVA GISWTLGRTYYADSVKGRFTISGDNAKNTVYLQMNSLKPEDTAVYYCAS DESDAYNYWGQGTQVTVSS
PDKKPMP13B4	1662	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKVREFVA AISWSLSRTHYADSVEGRFTISRDNAKNTAFMQMTSLKPEDTAVYYCAA APRPKWVSVRYFSTSSNYDYWGQGTQVTVSS
PDKKPMP13B5	1663	EVQLVESGGGLVQAGGSLRLSCTASGSAFSSSVVGWFRQAPGKEREFVT AINWSGARTDYADSVKGRFTISRDNAKNTAYLQMSSLKPEDTAVYYCAA GSDRYARVLTEYEYVWWGQGTQVTVSS
PDKKPMP13B11	1664	EVQLVKSGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKYVSVRYFSTSSNYDYWGQGTQVTVSS
PDKKPMP13E4	1665	EVQLVESGGGLVQPGGSLRLSCAASGFPFSIYPLKWVRQAPGKGLEWVS HISRDGGRTMYADSVKGRFTISRDNAKNMLYLQMNSLKPEDTAVYFCAR SAGKVVSRASLENDYRGQGTQVTVSS
PDKKPMP13H9	1666	EVQLVESGGGLVQAGGSLRLSCAASGLTFSTYAMGWFRQAPGKEREFVG AINWSSRSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GTAYVGGVRRRKAEYDYWGQGTQVTVSS

PDKKPMP13A2	1667	EVQLVESGGGWVQAGDSLRLSCTASGSAFSSSVVGWFRQAPGKEREFVT AINWSGARTYYVDSVKGRFTISRDNAKNTAYLQMNSVKPEDTAVYYCAA GRDARARALTEYEYVWWGQGTQVTVSS
PDKKPMP13A3	1668	EVQLVESGGGLVQAGGSLRLSCTASGSAFSSHVVGWFRQAPGKERDFVT AINWSGARTAYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYFCAA GRDRYARDLTEYEYLWWGQGTQVTVSS
PDKKPMP13A6	1669	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYGMGWFRHAPGKEREFVA GISWTLGRTYYADSVKGRFTISGDNAKNTVYLQMNSLKPEDTAVYYCAS DESDAANYWGQGTQVTVSS
PDKKPMP13A7	1670	EVQRVESGGGLVQAGGSLRLSCTASGSAFSSSVVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAS GRDRYARELSEYEYVWWGQGTQVTVSS
PDKKPMP13A8	1671	EVQLVESGGGLVQPGGSLRLSCAASGFPFSIYPMKWVRQAPGKGLEWVS HITRDGERTMYADSVKGRFTISRDNAKNMLYLQMNSLKPEDTAVYFCAR SAGKVVSRASLENDYRGQGTQVTVSS
PDKKPMP13A10	1672	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYGMGWFRPAPGKEREFVA GISWTLGRTYYADSVKGRFTISGDNAKNTVYLQMNSLKPEDTAVYYCAS DESDAYNYWGQGTQVTVSS
PDKKPMP17A4	1673	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYGMGWFRQAPGKEHEFVA GISWTLGRTYYADSVKGRFTISGDNAKNTVYLQMNSLKPEDTAVYYCAS DESDAYNYWGQGTQVTVSS
PDKKPMP17A5	1674	EVQLVESGGGLVQAGGSLRLSCTASGRTFDNYAMGWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKWVSVSYFSTTSNYDYWGQGTQVTVSS
PDKKPMP17A6	1675	EVQLVESGGGLVQAGGSLRLSCTASGRTFDNYAMGWFRQSPGKAREFVA AISWSLSRTHYADSVEGRFAISRDNAKNTVLLQMSGLKPEDTAVYYCAA APRPKRVSVSYFYTSSNYDYWGQGTQVTVSS
PDKKPMP17A7	1676	EVQLVESGRGLVQAGGSLRLSCTASGRAFSSTVVGWFRQAPGKERELVT AINWSGARTYYADSVKGRFTISRDNAKNSVYLQMNSLKPEDTAVYSCAA GSDRYARVLTEYEYVWWGQGTQVTVSS
PDKKPMP17A9	1677	EVQLVESGGGLVQAGGSLNLSCVLSGRTLWRSNMGWFRQAPGKEREFVG SIDWITTNIYYADSVKGRFTISRDNPKNTFYLQMNSLKPEDTAVYRCAS SGPADARNGDRWALWGQGTQVTVSS
PDKKPMP17A10	1678	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKWVSVRYFSNSSNYDYWGQGTQVTVSS
PDKKPMP17A12	1679	EVQLVESGGGLVQAGGSLRLSCTASGSAFSSSVVGWFRQAPGKEREFVT AINWSGARTYYADSVKGRFTISRENAKNTAYLQMDSLKPEDTAVYYCAA GSDRYARAFTDYEYVWWGQGTQVTVSS
PDKKPMP17B2	1680	KVQLVESGGGLVQAGGSLRLSCVLSGRTLSRSNMGWFRQAPGKEREFVA SIDWITINTYYADSVKGRFTISRDNPKNTFYLQMNSLKPEDTAVYRCAS SGPADARNGERWHLWGQGTQVTVSS
PDKKPMP17B4	1681	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKWVSVRYFSTSLNYDYWGQGTQVTVSS
PDKKPMP17B10	1682	KVQLVESGGGLVQPGGSLRLSCAASGFPFSIYPLKWVRQAPGKGLEWVS HISRDGGRTMYADSVKGRFTISRDNAKNMLYLQMNSLKPEDTAVYFCAR SAGKVVSRASLENDYRGQGTQVTVSS
PDKKPMP17G10	1683	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVG AISWSLSRTHYTDSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKSISVRYFSTSSNYEYWGQGTQVTVSS
PDKKPMP17A2	1684	KVQLVESGGGLVQAGGSLRLSCAASGHTFSSYAMGWFRQAPGKEREFVA AINWSSRSTVYADSVKGRFTISRDNAKNTVWLQMSSLKPEDTAVYYCAA GSAYVAGVRRRNAQYDYWGQGTQVTVSS
PDKKPMP19B10	1685	EVQLVESGGGLVQAGDSLTLSCTASGRTFDNYAMGWFRQAPGKEREFVA AISWSLSRTHYADYAKGRFTISRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKRISVSYFSTTSNYDYWGQGTQVTVSS
PDKKPMP19C1	1686	EVQLVESGGGLVQAGGSLRLSCTASGRTFDNHAMGWFRQAPGKEREFVA AISWSLSRTHYADSVSGRFTTSRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKRVSVSYFSTSSNYDYWGQGTQVTVSS

PDKKPMP19D2	1687	EVQLMESGGGLVQAGGSLTLSCTASGRTFDNYAMGWFRQAPGKEREFVA AISWSLSRTHYADSVLGRFTISRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKRISVSYFSTSSNYDYWGQGTQVTVSS
PDKKPMP19E1	1688	EVQLVESGGGLVQAGGSLNLSCTASGSAFSSSVVGWFRQAPGKEHEFVT GINWSGARTYYADSVKGRFTISRDNAKNTAYLQMHSLKPEDTAVYYCAS GRDRYARDLSEYEYVWWGQGTQVTVSS
PDKKPMP19F5	1689	EVQLVESGGGLVQAGGSLNLSCTASGNAFSSSVVGWFRQAPGKEHEFVT GINWSGARTYYADSVKGRFTISRDNAKNTAYLQMHSLKPEDTAVYYCAS GRDRYARDLSEYEYVWWGQGTQVTVSS
PDKKPMP19A3	1690	KVQLVESGGGPVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWYVSTTFYADSVKDRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKRISVRYFSTSSNYDYWGQGTQVTVSS
PDKKPMP19A8	1691	EVQLVESGGGLVQPGGSLRLSCAASGFPFSIYPMKWVRQAPGKGLEWVS HITRDGGRTMYADFVKGRFTISRDNAKNMLYLQMNSLKPEDTAVYFCAR SAGKVVSRASLENDYRGQGTQVTVSS
PDKKPMP19B4	1692	EVQLVESGGGLVQAGGSLRLSCTASGGAFSSTVVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTISRDNAKDTAYLEMNSLKPEDTAVYYCAS GRDRYARALPDYEYLWWGQGTQVTVSS
PDKKPMP19B7	1693	EVQLVESGGGLVQAGDSLTLSCTASGRTFDNYAMGWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKRVSVSYFSTTSNYDYWGQGTQVTVSS
PDKKPMP20A8	1694	EVQLVESGGGLVQPGGSLRPSCTASGSAFSGSVVGWFRQAPGKEREFVT AINWSGARTYYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAA ARDRYARALTEYEYLWWGQGTQVTVSS
PDKKPMP20A10	1695	EVQLVESGGGLVQAGGSLRLSCTASGRAFSSSVVGWFRRAPGKEREFVT GINWSGARAYYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAS GSDRFARELSEYDYVWWGQGTQVTVSS
PDKKPMP20A12	1696	EVQLVESGGGSVQAGGSLRLACTASGRPFDNYAMAWFRQAPGKEREFVA AVSWSLSRTHYADSVKGRFIISRDNAKNTAFMQMSSLEPEDTAVYYCAA APRPKWVSVRYFTTSSNYDYWGQGTQVTVSS
PDKKPMP20B1	1697	KVQLVESGGGLVQAGGSLRLSCVLSGRTLSRSNMGWFRQAPGKEREFVA SIDWITINTYYADSVKGRFTISRDNPKNTLYLQMNSLKPEDTAVYRCAS SGPADARNGERWALWGQGTQVTVSS
PDKKPMP20F10	1698	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYGMAWFRQAPGKEREFVA GISWTLGRTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAS DHYDTDTKSDVYNYWGQGTQVTVSS
PDKKPMP20H1	1699	EVQLVESGGGLVQAGGSLRLSCIASGRAFSSSVVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTVSRDNAKLTAYLQMNSLKPEDTAVYYCAS GSDSRARELSRYEYVWWGQGTQVTVSS
PDKKPMP20A1	1700	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYAESVEGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKWVSVRYFSTSSNYDYWGQGTQVTVSS
PDKKPMP20A2	1701	EVQLVESGGGSVQAGGSLRLSCTASGRPFDDYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDGAKNTAFMQMSSLKPEDTAVYYCAA APRPKWVSVQYFSTSANYEYWGQGTQVTVSS
PDKKPMP20A3	1702	EVQLVESGGGLVQVGDSLRLSCAVSGRTLSNAIMGWFRQAPGKERDFVA AISRSAGSLTWYPTSVKGRFTISRDNDKNTAYLQMNSLKPEDTAVYYCA AAYRFSDYSRPAGYDYWGQGTQVTVSS
PDKKPMP3A3	1703	EVQLVESGGGLVQAGGSLRLSCVLSGRTFSRSDMGWFRQAPGKEREFVA SIDWITINTYYADSVKGRFTISRDNPKNTFYLQMNSLKPEDTAVYHCAS SGPADARNVQRWALWCQGTQVTVSS
PDKKPMP3A6	1704	EVQLVESGGGLVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLTRTHYADSVKGRFTISRDNAKNTALMQMSSLKPEDTAVYYCAA APRPKWISVSYFSTSSNYDYWGQGTQVTVSS
PDKKPMP3A8	1705	EVQLVESGGGSVQAGGSLRLSCTASGRPFNNYAMAWFRQAPGKEREFVA AINWSLSRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKCVSVRYFSTSSNYDYWGQGTQVTVSS
PDKKPMP3A12	1706	EVQLVESGGGLVQAGGSLRLSCTASGRTFSTSAMAWFRQAPVKEREFVA AVIGSSGITDYADFVKGRFTIFIDNAKNTAYLQMNSLKPEDTAVYYCAA GFGSTSILRTQDDYDYWGQGTQVTVSS

PDKKPMP3B4	1707	EVQLVESGGGLVQPGGSLRLSCAASGFPFSIYPMKWVRQAPGKGLEWVS HITRDGGRTMYADSVKGRFTISRDNAMNMLYLQMNSLKPEDTAVYFCAR SAGKVVSRASLENDYRGQGTQVTVSS
PDKKPMP6A7	1708	EVQLMESGGGLVQAGGSLRLSCAASGRTFSRYAMGWFRQAPGKEREFVA AISWSGGSIYYADSVKGRFTVSRDNANNTVYLQMNSLKPEDTAVYYCAT DSARFSSGERRSRMQYDFWGQGTQVTVSS
PDKKPMP1A2	1709	EVQLVESGGGLVQAGGSLRLSCAASERTFTNYAMGWFRQAPGKEREFVA AISWSGESTLYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYFCAA GNGGRNYGHSRARYDYWGQGTQVTVSS
PDKKPMP4F8	1710	EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKERDGVS CISSSDGSTYYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAV EVPSSCGYYRGSYYYTDEEFAYWGQGTQVTVSS
PDKKPMP7A4	1711	EVQLVESGGGLVQAGGSLRLSCAASGFTFENYAMGWFRQAPGKEREGVS CISSSDGSTYYADSVKGRFTISSDNTKNTVYLQMNSLKPEDTAVYYCAA EVPSSCGYYSGNYDYTDDEYDFWGQGTQVTVSS
PDKKPMP7B12	1712	EVQLVESGGGLVQAGGSLRLSCAASGFTFENYAMGWFRQAPGKEREGVS CISSSDGRTYYADSVKGRFTISSDNTKNTVYLQMNSLKPEDTAVYYCAA EVPSSCGYYSGNYDYTDDEYDFWGQGTQVTVSS
PDKKPMP12G3	1713	EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKERDGVS CISSSDGSTYYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAV EVPSSCGYYRGSSYYTDEEFAYWGQGTQVTVSS
PDKKPMP12F12	1714	EVQLVESGGGLVQAGGSLRLSCAASGFTFENYAMGWFRQAPGKEPEGVS CISSSDGSTYYADSVKGRFTISSDNTKNTVYLQMNSLKPEDTAVYYCAA EVPSSCGYYSGNYDYTDDEYDFWGQGTQVTVSS
PDKKPMP14H2	1715	EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKERDGVS CISSSDGSTYYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAV EVPSSCGYYRGSSYYTDEELAYWGQGTQVTVSS
Ctr-1D11	1716	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYGMSWVRQAPGKGLEWVS RINSGGDIINYVGSVKGRFTISRDNYKNILYLQMNGLKPEDTAIYYCAK SRDIYGWSPKDYRGQGTQVTVSS
DKKPMP13-C11A08	1717	KVQLVESGGGLVQAGGSLNLSCAAHGHTFSSYAMGWFRQAPGKEREFVA AINWSSRSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GTAYVNGVRRRKAEYDYWGQGTQVTVSS
DKKPMP13-C2A02	1718	EVQLVESGGGLVEPGGSLRLSCAASGFPFSIYPMKWVRQAPGKGLEWVS HITRDGGRTMYADSVKGRFTISRDNAKNMLYLQMNSLKPEDTAVYFCAR SAGKVVSRASLENDYRGQGTQVTVSS
DKKPMP13-C6A05	1719	EVQLVESGGGSVQAGGSLTLSCTASGRPFDNYAMAWFRQAPGKEREFVA AVSWSLSRTHYADSVLGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKWVSVRYFSTSSNYDYWGQGTQVTVSS
DKKPMP13-C8A06	1720	KVQLVESGGGLVQAGGSLRLSCTASGSAFSSTVVGWFRQAPGKERDFVT AINWSGARTAYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAA GRDRYARDLTEYEYLWWGQGTQVTVSS
DKKPMP13-C9A07	1721	EVQLVESGGGLVQAGGSLRLSCTASGSAFSSSVVGWFRQAPRKEREFVT AINWSGARTDYADSVKGRFTISRDNAKNTAWLQMNSLKPEDTAVYYCAA GSDRYARVLTEYEYVWWGQGTQVTVSS
DKKPMP13-D5A12	1722	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYGMGWFRQAPGKEHEFVA GISWTLGRTYYADSVKGRFTISGDNAKNTVYLQMNSLKPEDTAVYYCAS NESDAYNYWGQGTQVTVSS
DKKPMP13-E6B04	1723	EVQLVESGGGLVQAGGSLTLSCTASGRTFDNYAMGWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTVLLQMSSLKPEDMAVYYCAA APRPKWISVSYFSTSSNYDYWGQGTQVTVSS
DKKPMP13-E9B06	1724	EVQLVESGGGLVQAGGSLRLSCTASGRAFSSSVVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAS GRDSRARALFEYEYVWWGQGTQVTVSS
DKKPMP13-F3B11	1725	EVQLVESGGGLVQAGGSLRLSCTASGSAFSSTVVGWFRQAPGKEREFVT AINWSGARTYYISSVKGRFTISRDNAKNTAYLQMDSLKPEDTAVYYCAA GSDRYARVLTEYDYVWWGQGTQVTVSS
DKKPMP13-F6B12	1726	EVQLVESGGGLVQAGGSLRLSCTASGRAFSSSVVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTISRDNGMNTAYLQMNRLKPEDTAAYYCAS GRDRYARALFEYDYVWWGQGTQVTVSS

DKKPMP13-F7C01	1727	EVQLVESGGGLVQAGGSLNLSCALSGRTFCRYSMGWFRQAPGKEREFVG SIDWIPANIYYADSVKGRFTISGDNPKNTLYLQMNSLKPEDTAVYHCAS NDPADARNGERGTLWGQGTQVTVSS
DKKPMP13-F8C02	1728	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVG AISWSLSRTNYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKWVSVRYFSTSSNYDYWGQGTQVTVSS
DKKPMP13-F9C03	1729	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKYVSVRYFSTSSNYDYWGQGTQVTVSS
DKKPMP13-G11C11	1730	EVQLVESGGGLVQAGGSLRLSCTASGRAFSTTVVGWFRQAPGKERELVT AINWSGARTYYADSVKGRFTISRDNAKNTVYTQMNSLKPEDTGVYYCAA GRDSRARVLTEYEYVWWGQGTQVTVSS
DKKPMP13-G1C04	1731	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYGMGWFRQAPGKEREFVA GISWTLGRTYYADSVKGRFTISGDNAKNTVYLQMNSLKPEDTAVYFCAS DESDAYNYWGQGTQVTVSS
DKKPMP13-G7C07	1732	EVQLVESGGGLVQAGGSLRLSCVASGHTFSSYAMGWFRQAPGKEREFVA AINWSSRSLVYADSVKGRFTISRDNAKNTVYLQMNSLKLEDTAVYYCAA GSAYVAGVRRRNAEYDSWGQGTQVTVSS
DKKPMP13-G9C09	1733	EVQQVESGGGLVQAGGSLRLSCTASGRAFSSTAVGWFRQAPGKERELVT AINWSGARTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GSDRYARVLTDYEYVWWGQGTQVTVSS
DKKPMP13-H10D07	1734	EVQLVESGGGLVRAGGSLRLSCAASGLTFSSYAMGWFRQAPGKEREFVA AVNWSGRRELYADSVKGRFTISRDNAKNTVYLQMDSLKPEDTAVLYCAA RENRYSFNYGDINQYNYWGQGTQVTVSS
DKKPMP13-H4D03	1735	KVQLVESGGGLVQPGGSLKLSCAASGFPFSIYPMKWVRQAPGKGLEWVS HITRDGGRTMYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYFCAR SAGQVVSRASLENDYRGQGTQVTVSS
DKKPMP17-C10E04	1736	EVQLVESGGGLVQAGGSLRLSCAASGDTFSNYAMGWFRQAPGKEREFVA AINWSSRSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GASYVAGVRRRNAEYLYWGQGTQVTVSS
DKKPMP17-C11E05	1737	EVQLVESEGGLVQAGGSLNLSCTASGSAFSSSVVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAS GRDRYARELSEYEYVWWGQGTQVTVSS
DKKPMP17-C1D09	1738	EVQLVESGGGLVQAGGSLRLSCTASGRAFSSSAVGWFRQAPGKERELAT AINWSGARTYYADSVKGRFTISRDNAKNTVYLQMNSLKLEDTAVYYCAA ASDRYARVLTEYEYVWWGQGTQVTVSS
DKKPMP17-C7E01	1739	EVQLVKSGGGLVQAGGSLRLSCTASGSAFSSTVVGWFRQAPGKERELVT AINWSGARTYYADSVKGRFTISRDNARNTAYLQMNSLKPEDTAVYYCAA GSDRYARVLTEYEYMWWGQGTQVTVSS
DKKPMP17-C8E02	1740	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAVAWFRQAPGKEREFVA AISWSLSRTHYANSVEGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKRVSVQYFSTSSNYDYWGQGTQVTVSS
DKKPMP17-D10E11	1741	EVQLVKSGGGLVQAGGSLRLSCAASGRTFSSYGMGWFRQAPGKEREFVA GISWTLGRTYYADSVKGRFTISGDNAKNTVYLQMNSLKPEDTAVYYCAS DESDAYNYWGQGTQVTVSS
DKKPMP17-D12E12	1742	EVQLVESGGGLVQAGDSLRLSCTASGSAFSRSVVGWFRQAPGKEREFVT AINWSGARTDYADSVKGRFTISRNNAENTAYLQMNSLKFEDTAVYYCAA GWDRYARVLTEYDYVWWGQGTQVTVSS
DKKPMP17-D9E09	1743	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYGMGWFRQAPGKEREFVA GISWTLGRTYYADSVKGRFTISGDNAKNTVYLQMNSLKPEDTAIYYCAS DESDAYNYWGQGTQVTVSS
DKKPMP17-E1F01	1744	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKDTAFMQMSSLKPEDTAVYYCAA APRPKWVSVRYFSTSLNYDYWGQGTQVTVSS
DKKPMP17-E4F03	1745	EVQLVESGGGLVQAGGSLTLSCTASGRTFDNYAMGWFRQAPGKEREFVA AINWSLSRTHYADYVKGRFTISRDNAKNTVLLQMNSLKPEDTAVYYCAS APHPKRISVSYFSTTSNYDYGGQGTQVTVSS
DKKPMP17-E5F04	1746	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLTRTHYAESVEGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKRISVRYFSTSSNYDYWGQGTQVTVSS

DKKPMP17-E6F05	1747	EVQLVESGGGLVQAGGSLRLSCTASGRAFSSSVVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTISRDNAKNTAHLQMNSLKREDTAVYYCAS GRDRYARVLPEYEYEWWGQGTQVTVSS
DKKPMP17-E8F07	1748	EVQPVESGGGLVQAGGSLRLSCTASGSAFSSTVVGWFRQAPGKEREIVT AINWSGARTYYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAA GSDRYARVLTEYEYVWWGQGTQVTVSS
DKKPMP17-F10G04	1749	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGEEREFVA AISWALTRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKWISVRYFSTSSNYDYWGQGTQVTVSS
DKKPMP17-F12G05	1750	EVQLVESGGGSVQAGGSLRLSCTAAGRPLDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKYVSVRYFSTSSNYDYWGQGTQVTVSS
DKKPMP17-F2F12	1751	EVQLVESGGGLVQAGGSLRLSCTASGSAFSSHVVGWFRQAPGKERDFVT AINWSGARTAYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAA GRDRYARDLTEYEYLWWGQGTQVTVSS
DKKPMP17-F3G01	1752	EVQLVQSGGGLVQAGGSLRLSCVLSGRTLWRSNMGWFRQAPGKEREFVG SIDWITTNTYYADSVKGRFTISRDNPKNTFFLQMNSLKPEDTAVYRCAS SGPADARNGERWALWGQGTQVTVSS
DKKPMP17-F4G02	1753	EVQLVESGGGLVQAGGSLTLPCTVSGRTFDNYAMGWFRQAPGKEREFVA AISWSLSRTHYADSAKGRFTISRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKRVSVSYFSTASNYDYWGQGTQVTVSS
DKKPMP17-G11H01	1754	EVQLVESGGGLVQAGGSLGLSCAASGHTFSSYAMGWFRQAPGKEREFVA AINWSSRSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCAA GSAYVAGVRRRNAQYDYWGQGTQVTVSS
DKKPMP17-G1G06	1755	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYADSVEGRFTISRDNAKNTAFMQMISLKPEDTAVYYCAA APRPKRVSVRYFSTSSNYDYWGQGTQVTVSS
DKKPMP17-G2G07	1756	EVQLVEPGGGLVQAGGSLRLSCTASGRAFSSTVVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAS GRDQYARALPEYEYVWWGQGTQVTVSS
DKKPMP17-G3G08	1757	KVQLVESGGGLVQAGDSLTLSCTASGRTFDNYAMGWFPQAPGKEREFVA AISWSLSRTHYADYVKGRFAISRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKRISVSYFSTTSNYDYWGQGTQVTVSS
DKKPMP17-G4G09	1758	EVQLVESGGGLVQAGGSLRLSCTASGSAFSSTVVGWFRQAPGKERELVT AINWSGARTYYADSVKGRFTISRDNARNTAYLQMNSLKPEDTAVYYCAA GSDRYARVLTEYEYMWWGQGTQVTVSS
DKKPMP17-G5G10	1759	EVQLVESGGGLVQAGGSLRLSCTASGRTFDNHAMGWFRQAPGKERELVA AISWSLSRTHYADSVKGRFTISRDNAKNTALLQMSSLKPEDTAVYYCAA APRPKYVSVSYFSTSSNYDYWGQGTQVTVSS
DKKPMP17-H5H04	1760	EVQLVESGGGLVRAGGSLRLSCAASGRTFSSYGMGWFRHAPGKEREFVA GISWTLGRTYYADSVKGRFTISGDNAKNTVYLQMNSLKPEDTAVYYCAS DESDAANYWGQGTQVTVSS
DKKPMP17-H6H05	1761	EVQLVESGGGSVQAGGSLRLFCTASGRPFDNYAMAWFRQAPGKEREFVA AISWYLTTTYYADSVKGRFTISRDNAKNTAFMQMSSLEPEDTAVYYCAA APRPTRVSVRYFSTRSNYNYWGQGTQVTVSS
DKKPMP17-H8H06	1762	EVQLVESGGGLGQPGGSLRLSCAASGRSFSTYTMGWFRQAPGKERQFIA AMRVGVTYYVDSVKGRFTISRDNAKNTVSLQMNSLKPEDTAVYYCAADF VLAPGMRVSRPPNYWGQGTQVTVSS
DKKPMP19-C3H07	1763	EVQLVESGGGSVQAGGSLRLSCTASGRPFDNYAMAWFRQAPGKEREFVA AISWSLSRTHYANSVEGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKRVSVQYFVTSSNYDYWGQGTQVTVSS
DKKPMP19-C6H10	1764	EVQLVESGGGLVQAGGSLRLSCTASGSAFSGSVVGWFRQAPGKEREFVT AINWSGARTYYVDSVKGRFTISRDNAKNTAHLQMNSLKPEDTAVYYCAA ARDRYARVLTEYEYLWWGQGTQVTVSS
DKKPMP19-C8H11	1765	EVQLVESGGGLVQSGGSLRLSCTASGRTFDNYAMGWFRQAPGKEREFVA AISWALSRTHYVDSVKGRFTISRDNAKDTVQLQMSSLKPEDTAVYYCAA APRPKRVSFSYFSTSSNYEYWGQGTQVTVSS
DKKPMP19-C9H12	1766	EVQLVESGGGSVQAGGSLRLSCTASGRALSSAVVGWFRQAPGKEREFVT AINWSGARTYYADSVKGRFTISRDNAKNTVYLQMDSLKSEDTAVYYCAS ASDRYARPLDEYEYVSWGQGTQVTVSS

DKKPMP19D12 A05	1767	EVQLVESGGGLVQAGGSLRLSCTASGRTFDNYAMGWFRQAPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKWVSVSYFSTTSNYEYWGQGTQVTVSS
DKKPMP19D5A 03	1768	EVQLVESGGGLVQAGGSLRLSCTASGRAFSSSVVGWFRQAPGKEREFVT GINWSGARTLYADSVKGRFTISRDNAKSTAFLQMNSLKPEDTAVYYCAS GRDRYARVLSEYEYEWWGQGTQVTVSS
DKKPMP19E11 A09	1769	EVQLVESGGGLVQAGGSLRLSCTASGRAFSSTVVGWFRQAPGKEREFVT AINWSGARTYYAESVKGRFTISRDNAKNSAYLQMNSLKPEDTAVYYCAA GSDRYARALTEYEYVWWGQGTQVTVSS
DKKPMP19E12 A10	1770	EVQLVESGGGSVQAGGSLKLSCTASGRPFNNYAMAWFRQAPGKEREFVA AISWSLSRTHYANSVKDRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKRVSVHYFSTSSNYDYGGQGTQVTVSS
DKKPMP19E3A 06	1771	EVQLVESGGGLVQAGDSLTLSCTASGRTFDNYAMGWFRQAPGKEREFVA AISWSLSRTHYADYVKGRFAISRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKRISVSYFSTTSNYDYWGQGTQVTVSS
DKKPMP19E7A 07	1772	EVQLVESGGGSMQAGGSLRLSCTASGRPFDNYAVAWFRQVPGKEREFVA AISWSLSRTHYANSVEGRFTISRDNAKNTAFMQMSSLKPEDTAVYYCAA APRPKRASVQYFSTSSNYDYWGQGTQVTVSS
DKKPMP19F12 B05	1773	EVQLVESGGGLVQPGGSLRLSCVASGFTFSMWPLKWVRQAPGKGLEWVS HITSNGDRTMYAASVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCAR SAGQVVSRASLENDYRGQGTQVTVSS
DKKPMP19F9B 03	1774	EVQLVESGGGLVQAGGSLRLSCVLSGRTLSRSNMGWFRQAPGKEREFVA SIDWITINTYYADSVKGRFTISRDNPKNTFYLQMNSLKPEDTAVYRCAS AGPADARNGERWALWGQGTQVTVSS
DKKPMP19G1B 06	1775	EVQLVESGGGLVQAGGSLRLSCTASGRTFDNYAMGWFRQVPGKEREFVA AISWSLSRTHYADSVKGRFTISRDNAKNTVLLQMSSLKPEDTAVYYCAA APRPKRVSVSYFSTTSNYDYWGQGTQVTVSS
DKKPMP19G6B 07	1776	EVQLVESGGGLVQAGGSLRLSCTASGRAFSSTVVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTISRDNAKNMAYLQMNSLKPEDTAVYYCAS GSDRYARALSEYEYVWWGQGTQVTVSS
DKKPMP19G9B 10	1777	EVQLVESGGGLVQAGGSLRLSCTASGRAFSSSVVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTISRDNAKNTAYLQMTSLKPEDTAVYYCAS GRDAYARVLPDYEYVWWGQGTQVTVSS
DKKPMP19H7C 06	1778	EVQLVESGGGLVQAGGSLRLSCTASGSAFSSTVVGWFRQAPGKEREFVT AINWSGARTYYADSVKGRFTISRDNAKNTGYLQMNSLKPEDTAVYYCAA GSDRYARVFTEYEYVWWGQGAQVTVSS
DKKPMP19H9C 07	1779	EVQLVESGGGLVQAGGSLRLSCTASGRALSSSVVGWFRQAPGKEREFVT GINWSGARTYYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAS GSDRYARVLSEYEYVWWGRGTQVTVSS
DKKPMP15G11	1780	EVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVG AINWSGGSTIYADSVKGRFTISRDNAENTVYLQMNSLKPEDTAVYYCAA GNGGRNYGHSRARYEYWGQGIQVTVSS
DKKPMP15H4	1781	QVQLVESGGGVVQAGDSLSLSCAASGRTFSSYAMGWFRQAPGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGRTYGHSRARYDYWGQGTQVTVSS
DKKPMP16A4	1782	EVQLVESGGGLVQAGGSLRLSCVVSGGTFSVYAMGWFRQAPGKLREIVA AISRTGSSIYYADSVKGRFTISRDDAKKTVYLQMNGLKSEDTAVYYCAV DGRANGERPAVAGYNYWGQGTQVTVSS
DKKPMP16A8	1783	QVQLVESGGGLVQAGGSLRLSCAASGRTFSTYAMGWFRQAPGKERELVA AISGSGGSTYYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTALYYCAA GNGGRHYGHSRARYDYWGQGTQVTVSS
DKKPMP14B11	1784	EVQLVESGGGLVQAGGSLRLSCAASGFTFENYAMGWFRQAPGKEPEGVS CISSSDGSTYYADSVKGRFTISSDNTKNTVYLQMNNLKPEDTAVYYCAA EVPSSCGYYSGNYDYTDDEYDFWGQGTQVTVSS
DKKPMP13B12	1785	EVQLVESGGGLVQAGGSLRLSCTASGSAFSSTVVGWFRQAPGKEREFVT AINWSGARTYYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAA GSDRYARVLTEYEYVWWGQGTQVTVSS
DKKPMP13E3	1786	EVQLVESGGGLVQAGGSLRLSCAASGGTFSSYAMGWFRQAPGKEREFVA AINWSSRSTVYADAVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GSAYVAGVRRRNAEYHYWGQGTQVTVSS

DKKPMP19B3	1787	EVQLVESGGGLVQAGDSLRLSCTASGSAFSSSVVGWFRQAPGKEREFVT AINWSGARTDYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAA GSDRYARVLTEYEYVWWGQGTQVTVSS
DKKPMP15A1	1788	EVQLVESGGGLVQAGGSLRLSCAVSGRTFSSYTMGWFRQAPGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAT DESRLYGGKRPKMLYDYWGQGTQVTVSS
DKKPMP15A5	1789	EVQLVESGGGLVQAGGSLRLSCAASGRAFSNYAMGWFRQAPGKEREFVA AISWSGGSAVYSDSVKGRFTISRDVAKNTMYLQMNSLKPEDTAVYYCAA GNGGRQYGHSRARYDSWGQGTQVTVSS
DKKPMP10D7	1790	EVQLVESGGGLVQAGGSLRLSCVVSGGTFSVYAMAWFRQAPGKLREIVA AISRTGSSIYYTDSVKGRFTISRDDAKKTVYLQMNSLKSEDTAVYYCAV DGRANGERPAVAGYNYWGQGTQVTVSS
DKKPMP10F12	1791	EVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGNEREFVA AISWSGGSTVYADSVKGRFTISRDNAENTVYLQMNSLKPEDTAVYYCAA GNGGSYYGHSRARYDYWGQGTQVTVSS
DKKPMP10G1	1792	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRLAPGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGRTYGHSRARYDYWGQGTQVTVSS
DKKPMP10G8	1793	EVQLVESGGGLVQAGGSLRLSCAASGRTISNAAMGWFRQAPGKEREFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSPKPEDTAVYYCAA GNGGRNYGHSRARYDYWGQGTQVTVSS
DKKPMP15A2	1794	EVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVA AISWSGASTIYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGSRYGHSRARYDYWGQGTQVTVSS
DKKPMP15B4	1795	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVA AISSSGSSTVYADSVKGRFAISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGRNYGHSRARYDYWGQGTQVTVSS
DKKPMP15G1	1796	KVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKERDFVA AISWSGGSTVYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA GNGGSYYGHSRARYDYWGQGTQVTVSS
DKKPMP6C9C11	1797	EVQLVESGGGLVQAGGSLRLSCTASGSAFRSTVVGWFRQAPGKERDLVT AINWSGARTYYADSVKGRFTISRDNAKNTAYLQMNSLKPEDTAVYYCAA GRDSYARVLTEYEYVWWGQGTQVTVSS

Table A-2: Amino acid sequences of humanized Nanobodies® that bind Dkk-1

Nanobody®	SEQ ID NO	Sequence
4E4basic	1798	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQTPGKGREGVS CISSSDGSTYYADSVKGRFTISSDNAKNTLYLQMNSLRPEDTAVYYCAA EVPSSCGGYSGSYYYTDDELDFWGQGTLVTVSS
4E4hum2	1799	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQAPGKGREGVS CISSSDGSTYYADSVKGRFTISSDNAKNTLYLQMNSLRPEDTAVYYCAA EVPSSCGGYSGSYYYTDDELDFWGQGTLVTVSS
4E4hum3	1800	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQTPGKGREGVS CISSSDGSTYYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAA EVPSSCGGYSGSYYYTDDELDFWGQGTLVTVSS
4E4hum5	1801	EVQLLESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQTPGKGREGVS CISSSDGSTYYADSVKGRFTISSDNAKNTLYLQMNSLRPEDTAVYYCAA EVPSSCGGYSGSYYYTDDELDFWGQGTLVTVSS
4E4hum6	1802	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQTPGKGREGVS SISSSDGSTYYADSVKGRFTISSDNAKNTLYLQMNSLRPEDTAVYYCAA EVPSSSGGYSGSYYYTDDELDFWGQGTLVTVSS
4E4hum1	1803	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAIGWFRQTPGKGREGVS CISSSDGSTYYADSVKGRFTISSDNAKNTLYLQMNSLRPEDTAVYYCAA EVPSSCGGYSGSYYYTDDELDFWGQGTLVTVSS
4E4hum4	1804	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQTPGKGREGVS CISSSDGSTYYADSVKGRFTISSDNSKNTLYLQMNSLRPEDTAVYYCAA EVPSSCGGYSGSYYYTDDELDFWGQGTLVTVSS
4E4hum7	1805	EVQLLESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQAPGKGREGVS CISSSDGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTAVYYCAA EVPSSCGGYSGSYYYTDDELDFWGQGTLVTVSS
4E4hum8	1806	EVQLLESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQAPGKGREGVS CISSSDGSTYYADSVKGRFTISSDNSKNTLYLQMNSLRPEDTAVYYCAA EVPSSCGGYSGSYYYTDDELDFWGQGTLVTVSS
16A12basic	1807	EVQLVESGGGLVQPGGSLRLSCAASGRTFNNYAMGWFRQTPGKGREFVA AISWIIGSTYYADSVKGRFTISRDNAKNTLYLQMNGLRPEDTAVYYCAA EGGYSGTYYYTGDFDSWGQGTLVTVSS
16A12hum1	1808	EVQLVESGGGLVQPGGSLRLSCAASGRTFNNYAMGWFRQTPGKGREFVA AISWIIGSTYYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAA EGGYSGTYYYTGDFDSWGQGTLVTVSS
16A12hum2	1809	EVQLVESGGGLVQPGGSLRLSCAASGRTFNNYAMGWFRQAPGKGREFVA AISWIIGSTYYADSVKGRFTISRDNAKNTLYLQMNGLRPEDTAVYYCAA EGGYSGTYYYTGDFDSWGQGTLVTVSS
16A12hum3	1810	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMGWFRQTPGKGREFVA AISWIIGSTYYADSVKGRFTISRDNAKNTLYLQMNGLRPEDTAVYYCAA EGGYSGTYYYTGDFDSWGQGTLVTVSS
16A12hum4	1811	EVQLVESGGGLVQPGGSLRLSCAASGRTFNNYAMGWFRQTPGKGREFVS AISWIIGSTYYADSVKGRFTISRDNAKNTLYLQMNGLRPEDTAVYYCAA EGGYSGTYYYTGDFDSWGQGTLVTVSS
16A12hum5	1812	EVQLLESGGGLVQPGGSLRLSCAASGRTFNNYAMGWFRQTPGKGREFVA AISWIIGSTYYADSVKGRFTISRDNAKNTLYLQMNGLRPEDTAVYYCAA EGGYSGTYYYTGDFDSWGQGTLVTVSS
16A12hum6	1813	EVQLVESGGGLVQPGGSLRLSCAASGRTFNNYAMGWFRQTPGKGREFVA AISWIIGSTYYADSVKGRFTISRDNSKNTLYLQMNGLRPEDTAVYYCAA EGGYSGTYYYTGDFDSWGQGTLVTVSS
16A12hum9	1814	EVQLLESGGGLVQPGGSLRLSCAASGRTFNNYAMGWFRQAPGKGREFVA AISWIIGSTYYADSVKGRFTISRDNSKNTLYLQMNGLRPEDTAVYYCAA EGGYSGTYYYTGDFDSWGQGTLVTVSS
16A12hum7	1815	EVQLLESGGGLVQPGGSLRLSCAASGRTFNNYAMGWFRQAPGKGREFVA AISWIIGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTAVYYCAA EGGYSGTYYYTGDFDSWGQGTLVTVSS

16A12hum8	1816	EVQLLESGGGLVQPGGSLRLSCAASGRTFNNYAMGWFRQTPGKGREFVA AISWIIGSTYYADSVKGRFTISRDNSKNTLYLQMNGLRPEDTAVYYCAA EGGYSGTYYYTGDFDSWGQGTLVTVSS

Table A-3: Amino acid sequences of polypeptides, compound or constructs that bind Dkk-1

Construct	SEQ ID NO	Sequence
16A12WT-9GS-ALB8	1817	EVQLVESGGGLVQAGGSLRLSCAASGRTFNNYAMGWFRQTPGKEREFVAAI SWIIGSTYYADSVKGRFTISRDNAKNTVYLQMNGLKPEDTAVYYCAAEGGY SGTYYYTGDFDSWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLR LSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTI SRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
16A12hum7-9GS-ALB8	1818	EVQLLESGGGLVQPGGSLRLSCAASGRTFNNYAMGWFRQAPGKGREFVAAI SWIIGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTAVYYCAAEGGY SGTYYYTGDFDSWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLR LSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTI SRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
4E4WT-9GS-ALB8	1819	EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQTPGKEREGVSCI SSSDGSTYYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAAEVPS SCGGYSGSYYYTDDELDFWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQ PGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSV KGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
4E4hum7-9GS-ALB8	1820	EVQLLESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQAPGKGREGVSCI SSSDGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTAVYYCAAEVPS SCGGYSGSYYYTDDELDFWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQ PGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSV KGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS

Table A-4: Linker sequences

Linker	SEQ ID NO:	Sequences
5GS	1821	GGGGS
7GS	1822	SGGSGGS
9GS	1823	GGGGSGGGS
10GS	1824	GGGGSGGGGS
15GS	1825	GGGGSGGGGSGGGGS
18GS	1826	GGGGSGGGGSGGGGGGGS
20GS	1827	GGGGSGGGGSGGGGSGGGGS
25GS	1828	GGGGSGGGGSGGGGSGGGGSGGGGS
30GS	1829	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
35GS	1830	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS

Table C-1. Immunization Schedules

Day	Llama 073	Llama 074	Llama 075	Tissue collection
	Human Dkk-1	Human Dkk-1	Mouse Dkk-1	
0	**27 ug**	**67 ug**	100 ug	10 ml pre-immune blood
7	100 ug	**250 ug**	100 ug	-
14	100 ug	**250 ug**	50 ug	-
21	50 ug	**125 ug**	50 ug	-
28	50 ug	**125 ug**	50 ug	10 ml immune blood
35	50 ug	**125 ug**	50 ug	-
39				150 ml immune blood PBL1 + lymph node bow biopsy (llama 075)
40	50 ug	**125 ug**		
43				150 ml immune blood PBL2 (llama 075)
46				150 ml immune blood PBL1 + lymph node bow biopsy (llama 073+074)
50				150 ml immune blood PBL2 (llama 073+074)

Table C-2. Composition of Nanobody® libraries

Library	Size (no. of clones)	Insert Efficiency (%)
073	7.5×10^7	100
074	5×10^7	100
075	7×10^7	96

Table C-3. Nanobody® selection conditions

Library	Selection conditions, Round 1	Selection conditions, Round 2
073, 074 and 075	10nM, 1nM, 0.1nM, 0.01nM biotinylated human or mouse Dkk-1 in solution, followed by capture with streptavidin beads, washing and trypsin elution of phages	1nM, 0.1nM biotinylated mouse Dkk-1 or 1nM, 0.1nM human DKK-1 in solution, followed by capture with streptavidin beads, washing and trypsin elution of phages

Table C-4: IC_{50} values for monovalent wild-type Nanobodies® for blocking the interaction of human Dkk-1 with the LRP5 receptor (FACS competition assay)

Nanobody®	IC_{50} (nM)
4E4	0.99
7B12	3.11
10A5	1.18
16A12	1.83
18B5	5.35

Table C-5: IC50 values for humanized bi-specific Nanobodies® for blocking the interaction of human Dkk-1 with the LRP5 receptor (FACS-based competition assay)

Nanobody®	IC_{50} (nM)
4E4wt	6.01
4E4wt-ALB8	5.03
4E4H7-ALB8	3.81
16A12wt	3.81
16A12wt-ALB8	9.82
16A12H7-ALB8	10.3

Table C-6

	Llama/immunization	Selection R1	Selection R2
10A5	73 / human DKK1	1nM human DKK1	1nM mouse DKK1
16A12	73 / human DKK1	1nM mouse DKK1	
18B5	73 / human DKK1	0.1nM mouse DKK1	
4E4	75 / mouse DKK1	0.1nM human DKK-1	
7B12	75 / mouse DKK1	10nM humanDKK1	
14A11	75 / mouse DKK1	1nM human DKK1	1nM mouse DKK1
14B11	75 / mouse DKK1	1nM human DKK1	1nM mouse DKK1
7A4	75 / mouse DKK1	10nM human DKK1	
12B11	75 / mouse DKK1	1nM mouse DKK1	
14A3	75 / mouse DKK1	1nM human DKK1	1nM mouse DKK1
14H2	75 / mouse DKK1	1nM human DKK1	1nM mouse DKK1
1B9	73 / human DKK1	0.1nM human DKK1	

CLAIMS

1. An amino acid sequence directed against and/or specifically binding Dkk-1,
   - that inhibits and/or blocks binding of LRP5/6 to Dkk-1;
   - that inhibits and/or blocks binding of BHQ880 to Dkk-1;
   - that inhibits and/or blocks binding of RH2-18 to Dkk-1;
   - that inhibits and/or blocks binding of Kremen to Dkk-1;
   - that neutralizes the inhibitory effect of Dkk-1 on Wnt-mediated signal transduction; and/or
   - that neutralizes the inhibitory effect of Dkk-1 on osteoblastic cell differentiation.

2. An amino acid sequence according to claim 1, that comprises one or more stretches of amino acid residues chosen from the group consisting of:
   a) the amino acid sequences of SEQ ID NO's: 335-543;
   b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 335-543;
   c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 335-543;
   d) the amino acid sequences of SEQ ID NO's: 753-961;
   e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 753-961;
   f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 753-961;
   g) the amino acid sequences of SEQ ID NO's: 1171-1379;
   h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;
   i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 1171-1379;

   or any suitable combination thereof.

3. An amino acid sequence according to any of claims 1 or 2, that cross-blocks the binding of at least one of the amino acid sequences of SEQ ID NO's: 1589-1797 to Dkk-1 and/or that is cross-blocked from binding to Dkk-1 by at least one of the amino acid sequences of SEQ ID NO's: 1589-1797.

4. An amino acid sequence according to any of claims 1 to 3, that essentially consists of a Nanobody® that

i) has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1 to 22 and/or SEQ ID NO's: 1589-1816, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

5. An amino acid sequence according to claim 4, that essentially consists of a partially or fully humanized Nanobody®.

6. An amino acid sequence according to any of claims 4 or 5, that is chosen from the group consisting of SEQ ID NO's: 1589-1816 or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with at least one of the amino acid sequences of SEQ ID NO's: 1589-1816.

7. Polypeptide that comprises or essentially consists of one or more amino acid sequences according to any of claims 1 to 6, and optionally further comprises one or more peptidic linkers.

8. Polypeptide according to claim 7, which is a multiparatopic construct, such as a biparatopic or triparatopic construct, that comprises at least one amino acid sequence directed against a first antigenic determinant, epitope, part or domain of Dkk-1 and at least one amino acid sequence directed against a second antigenic determinant, epitope, part or domain of Dkk-1 different from the first antigenic determinant, epitope, part or domain.

9. Polypeptide according to claims 8, wherein said polypeptide is directed against
- the LRP5/6 binding site on Dkk-1;
- the BHQ880 binding site on Dkk-1;
- the RH2-18 binding site on Dkk-1; and/or
- the Kremen binding site on Dkk-1.

10. Polypeptide according to any of claims 8 or 9, which can simultaneously bind the LRP5/6 binding site on Dkk-1 and to the Kremen binding site on Dkk-1 and/or which inhibits and/or blocks binding of LRP5/6 and Kremen to Dkk-1.

11. Compound or construct, that comprises or essentially consists of one or more amino acid sequences according to any of claims 1 to 6, and/or one or more polypeptides according to any of claims 7 to 10, and optionally further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers.

12. Compound or construct according to claim 11, which has an increased half-life, compared to the corresponding amino acid sequence according to any of claims 1 to 4 per se, Nanobody according to any of claims 5 or 6 or polypeptide according to any of claims 7 to 10 per se, respectively.

13. Compound or construct that comprises or that is chosen from the group consisting of SEQ ID NO's: 1817-1820 or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with at least one of the amino acid sequences of SEQ ID NO's: 1817-1820.

14. Monovalent construct, comprising or essentially consisting of one amino acid sequence according to any of claims 1 to 6.

15. Use of a monovalent construct according to claim 14, wherein the monovalent construct is used as a binding domain or binding unit in preparing a polypeptide according to any of claims 7 to 10 or a compound according to any of claims 11 to 13.

16. Nucleic acid or nucleotide sequence, that encodes an amino acid sequence according to any of claims 1 to 6, a polypeptide according to any of claims 7 to 10, a compound or construct according to any of claims 11 to 13, that is such that it can be obtained by expression of a nucleic acid or nucleotide sequence encoding the same, or a monovalent construct according to claim 14.

17. Host or host cell that expresses, or that under suitable circumstances is capable of expressing, an amino acid sequence according to any of claims 1 to 6, a polypeptide according to any of claims 7 to 10, a compound or construct according to any of claims 11 to 13 that is such that it can be

obtained by expression of a nucleic acid or nucleotide sequence encoding the same, or a monovalent construct according to claim 14; and/or that comprises a nucleic acid or nucleotide sequence according to claim 16.

18. Composition comprising at least one amino acid sequence according to any of claims 1 to 6, polypeptide according to any of claims 7 to 10, compound or construct according to any of claims 11 to 13, monovalent construct according to claim 14, or nucleic acid or nucleotide sequence according to claims 16.

19. Composition according to claim 18, which is a pharmaceutical composition, that further comprises at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and that optionally comprises one or more further pharmaceutically active polypeptides and/or compounds.

20. Method for producing an amino acid sequence according to any of 1 to 6, a polypeptide according to any of claims 7 to 10, a compound or construct according to any of claims 11 to 13 that is such that it can be obtained by expression of a nucleic acid or nucleotide sequence encoding the same, or a monovalent construct according to claim 14, said method at least comprising the steps of:

a) expressing, in a suitable host cell or host organism or in another suitable expression system, a nucleic acid or nucleotide sequence according to claim 16;

or

cultivating and/or maintaining a host or host cell according to claim 17 under conditions that are such that said host or host cell expresses and/or produces at least one amino acid sequence according to any of claims 1 to 6, polypeptide according to any of claims 7 to 10, compound or construct according to any of claims 11 to 13, or monovalent construct according to claim 14;

optionally followed by:

b) isolating and/or purifying the amino acid sequence according to any of claims 1 to 6, the polypeptide according to any of claims 7 to 10, the compound or construct according to any of claims 11 to 13, or the monovalent construct according to claim 14, thus obtained.

21. Method for preparing and/or generating a multiparatopic, such as biparatopic polypeptide according to any of claims 8 to 10, said method comprising at least the steps of:

a) providing a set, collection or library of nucleic acid sequences, in which each nucleic acid sequence in said set, collection or library encodes a fusion protein that comprises a first

amino acid sequence that can bind to and/or has affinity for a first antigenic determinant, part, domain or epitope on Dkk-1 that is fused (optionally via a linker sequence) to a second amino acid sequence, in which essentially each second amino acid sequence (or most of these) is a different member of a set, collection or library of different amino acid sequences;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on Dkk-1 different from the first antigenic determinant, part, domain or epitope on Dkk-1;

and

c) isolating the nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for a second antigenic determinant, part, domain or epitope on Dkk-1 different from the first antigenic determinant, part, domain or epitope on Dkk-1, obtained in b), optionally followed by expressing the encoded amino acid sequence.

22. Method for preparing and/or generating a biparatopic polypeptide according to any of claims 8 to 10, said method comprising at least the steps of linking two or more monovalent constructs according to claim 14 and for example one or more linkers.

23. Method for the prevention and/or treatment of at least one Dkk-1-associated disease or disorder, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one amino acid sequence according to any of claims 1 to 6, polypeptide according to any of claims 7 to 10, compound or construct according to any of claims 11 to 13, monovalent construct according to claim 14, or composition according to any of claims 18 or 19.

24. Use of an amino acid sequence according to any of claims 1 to 6, a polypeptide according to any of claims 7 to 10, a compound or construct according to any of claims 11 to 13, or a monovalent construct according to claim 14 in the preparation of a pharmaceutical composition for prevention and/or treatment of at least one Dkk-1 -associated disease or disorder; and/or for use in the method of claim 23.

25. Amino acid sequence according to any of claims 1 to 6, polypeptide according to any of claims 7 to 10, compound or construct according to any of claims 11 to 13, monovalent construct according to claim 14, or composition according to any of claims 18 or 19 for the prevention and/or treatment of at least one Dkk-1 -associated disease or disorder.

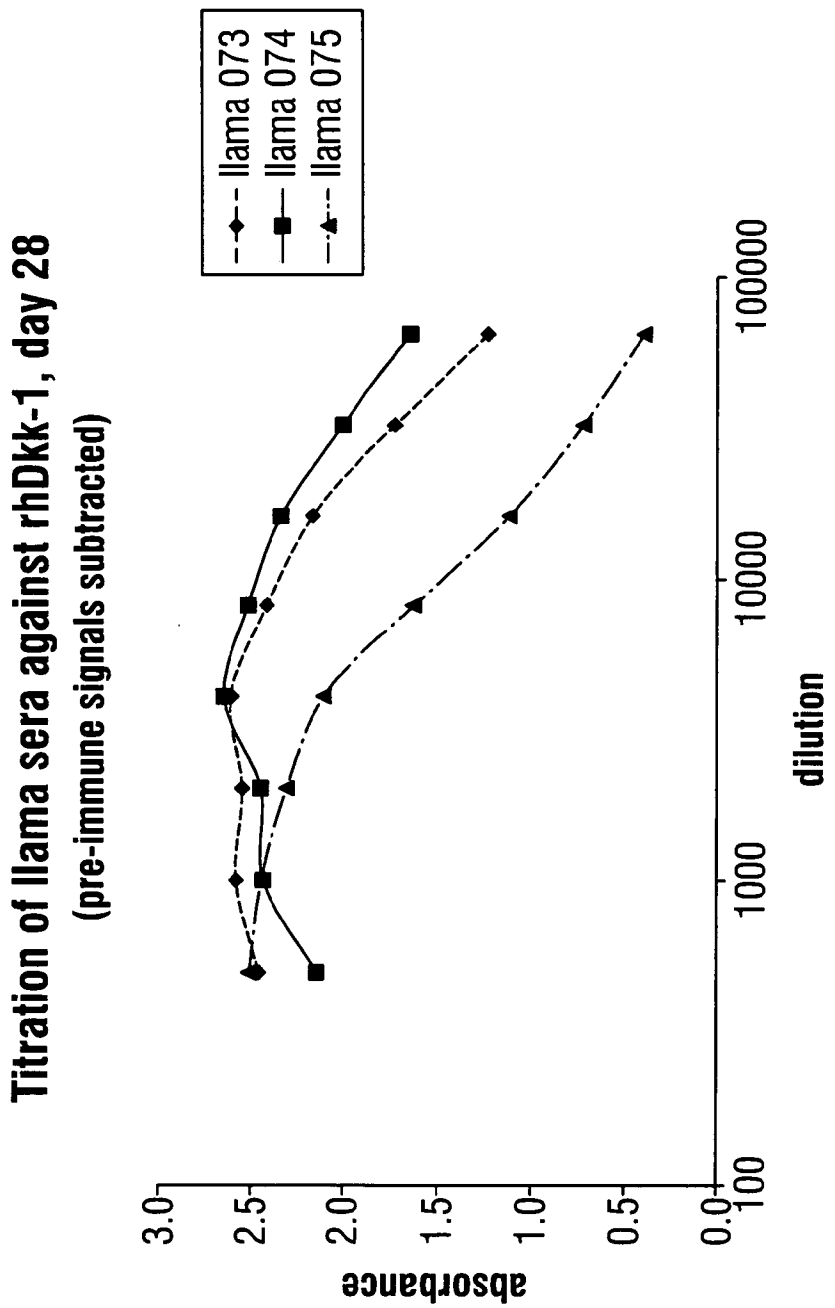

Titration of llama sera against rhDkk-1, day 28
(pre-immune signals subtracted)
llama 073
llama 074
llama 075
absorbance
3.0
2.5
2.0
1.5
1.0
0.5
0.0
100
1000
10000
100000
dilution

Figure 1A

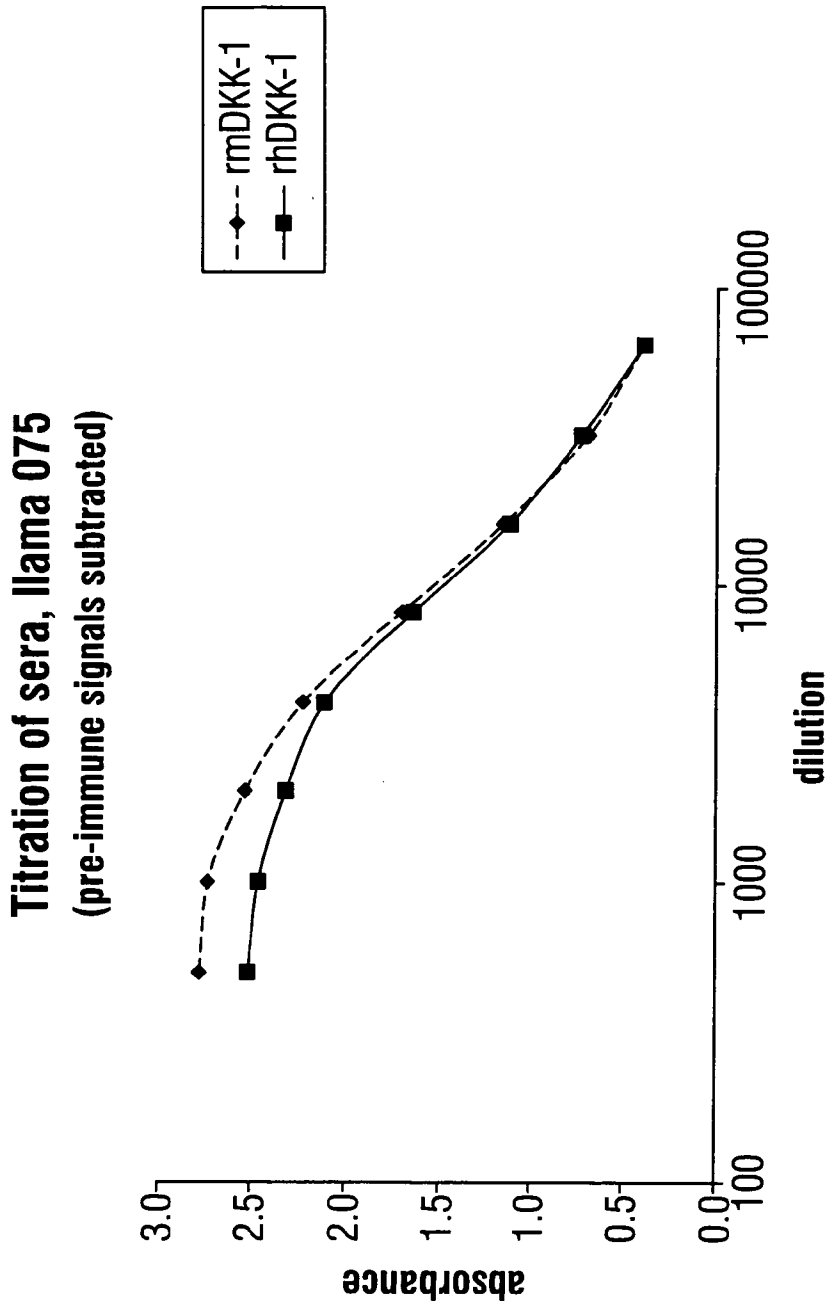

Titration of sera, llama 075
(pre-immune signals subtracted)
rmDKK-1
rhDKK-1
absorbance
3.0
2.5
2.0
1.5
1.0
0.5
0.0
100
1000
10000
100000
dilution

Figure 1B

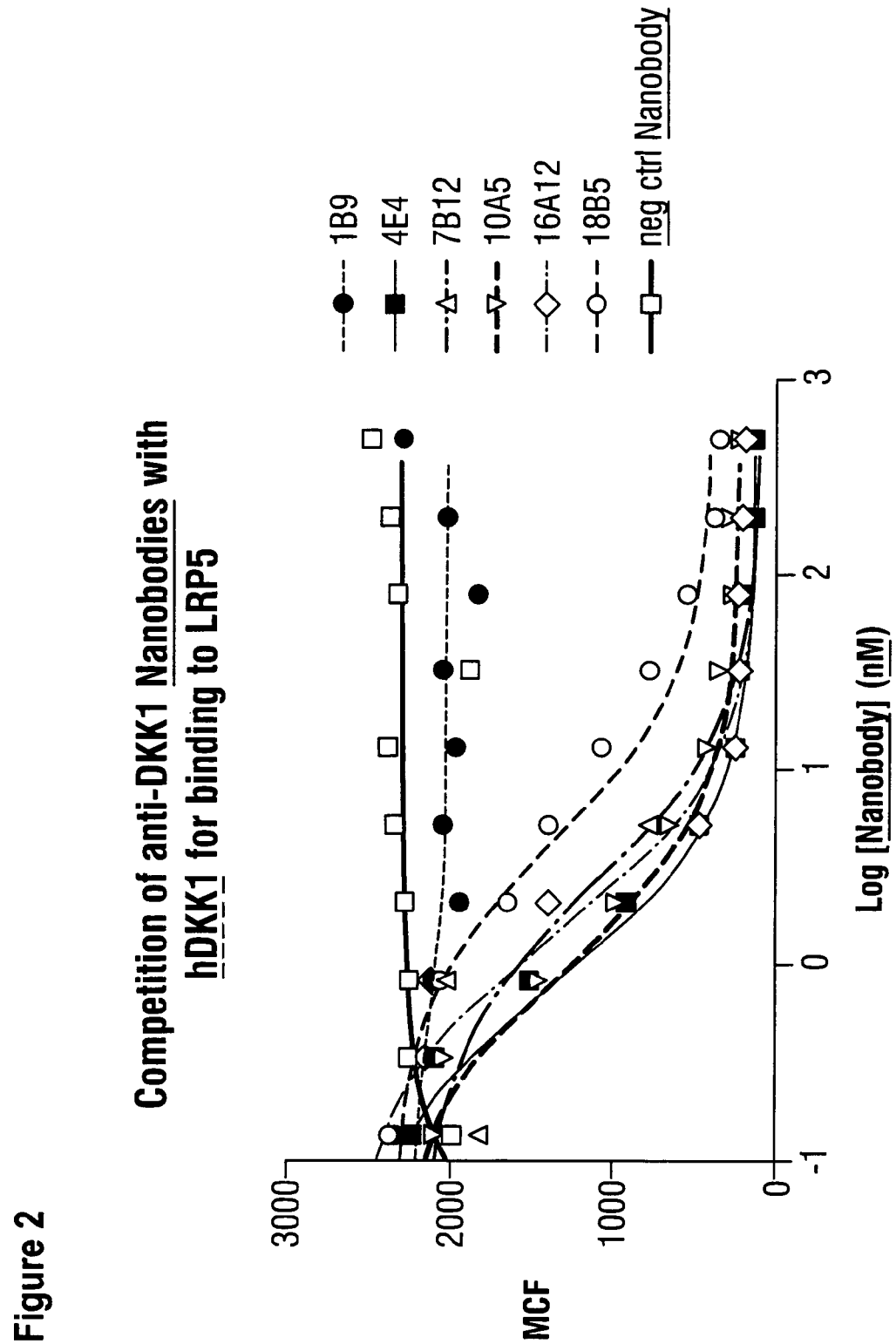
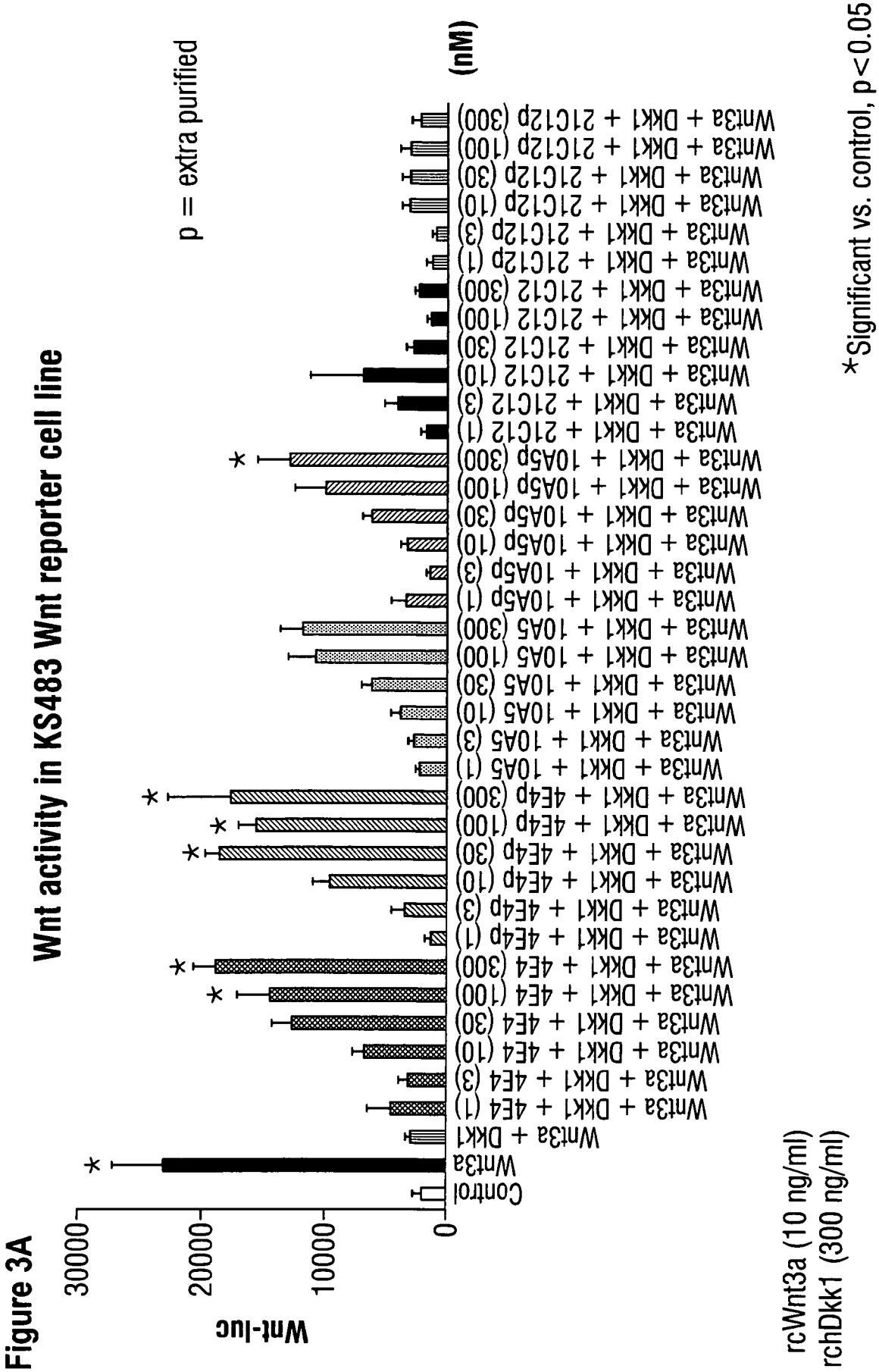

Competition of anti-DKK1 Nanobodies with hDKK1 for binding to LRP5
1B9
4E4
7B12
10A5
16A12
18B5
neg ctrl Nanobody
MCF
3000
2000
1000
0
-1
0
1
2
3
Log [Nanobody] (nM)

Figure 2


Figure 3A
Wnt activity in KS483 Wnt reporter cell line
Wnt-luc
30000
20000
10000
0
p = extra purified
(nM)
Control
Wnt3a
Wnt3a + Dkk1
Wnt3a + Dkk1 + 4E4 (1)
Wnt3a + Dkk1 + 4E4 (3)
Wnt3a + Dkk1 + 4E4 (10)
Wnt3a + Dkk1 + 4E4 (30)
Wnt3a + Dkk1 + 4E4 (100)
Wnt3a + Dkk1 + 4E4 (300)
Wnt3a + Dkk1 + 4E4p (1)
Wnt3a + Dkk1 + 4E4p (3)
Wnt3a + Dkk1 + 4E4p (10)
Wnt3a + Dkk1 + 4E4p (30)
Wnt3a + Dkk1 + 4E4p (100)
Wnt3a + Dkk1 + 4E4p (300)
Wnt3a + Dkk1 + 10A5 (1)
Wnt3a + Dkk1 + 10A5 (3)
Wnt3a + Dkk1 + 10A5 (10)
Wnt3a + Dkk1 + 10A5 (30)
Wnt3a + Dkk1 + 10A5 (100)
Wnt3a + Dkk1 + 10A5 (300)
Wnt3a + Dkk1 + 10A5p (1)
Wnt3a + Dkk1 + 10A5p (3)
Wnt3a + Dkk1 + 10A5p (10)
Wnt3a + Dkk1 + 10A5p (30)
Wnt3a + Dkk1 + 10A5p (100)
Wnt3a + Dkk1 + 10A5p (300)
Wnt3a + Dkk1 + 21C12 (1)
Wnt3a + Dkk1 + 21C12 (3)
Wnt3a + Dkk1 + 21C12 (10)
Wnt3a + Dkk1 + 21C12 (30)
Wnt3a + Dkk1 + 21C12 (100)
Wnt3a + Dkk1 + 21C12 (300)
Wnt3a + Dkk1 + 21C12p (1)
Wnt3a + Dkk1 + 21C12p (3)
Wnt3a + Dkk1 + 21C12p (10)
Wnt3a + Dkk1 + 21C12p (30)
Wnt3a + Dkk1 + 21C12p (100)
Wnt3a + Dkk1 + 21C12p (300)
rcWnt3a (10 ng/ml)
rchDkk1 (300 ng/ml)
*Significant vs. control, p<0.05

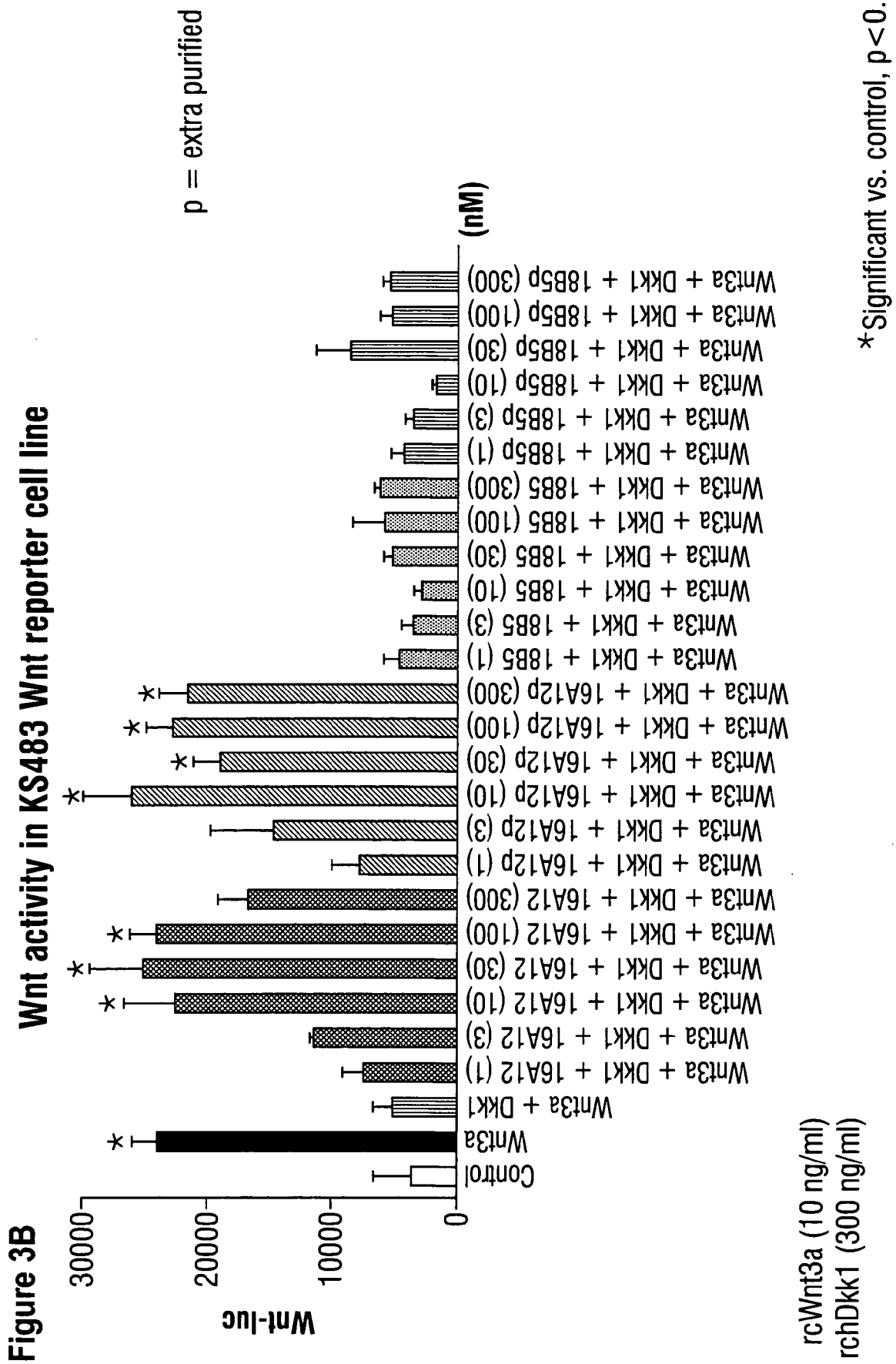
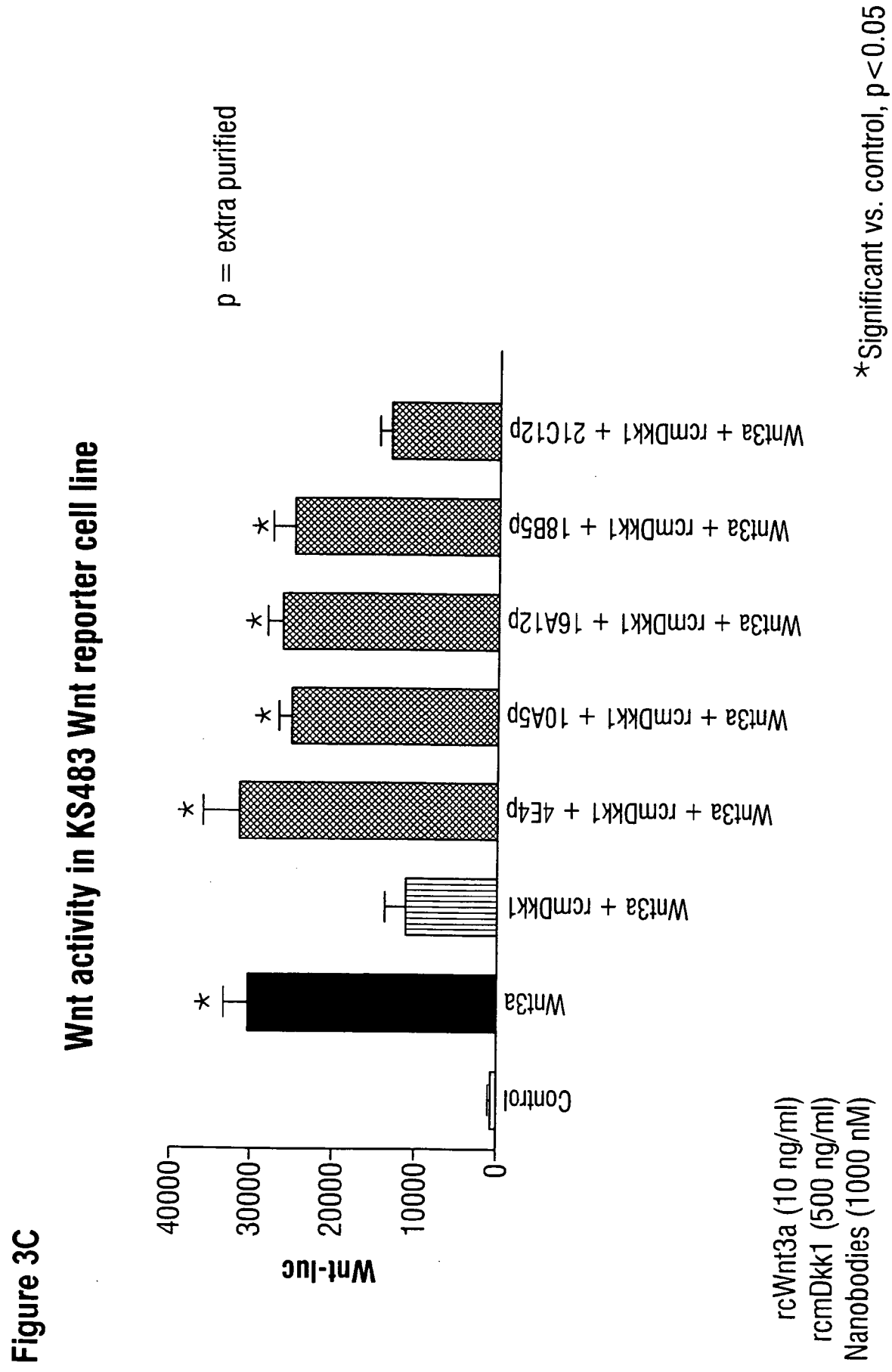
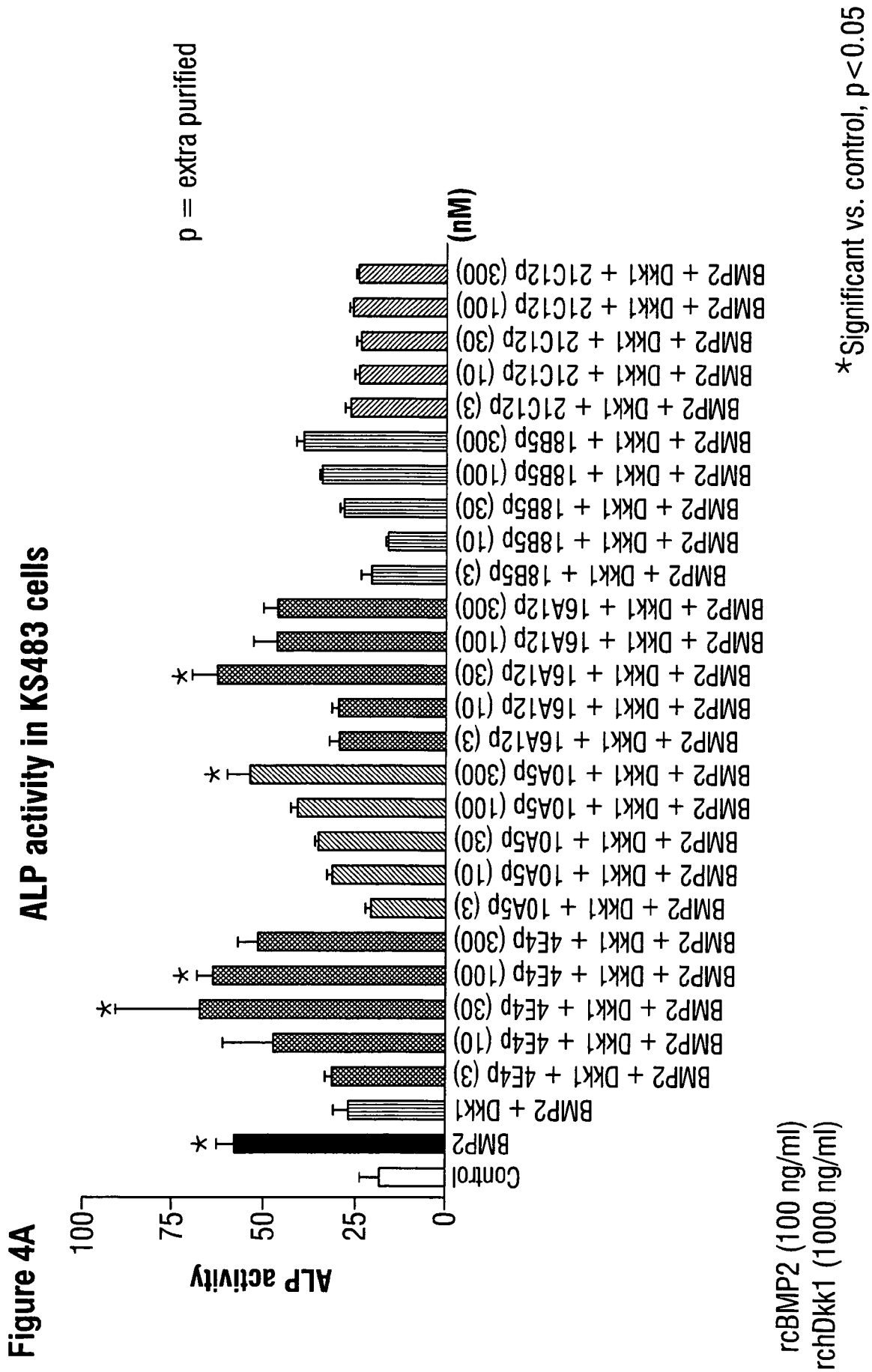

Wnt activity in KS483 Wnt reporter cell line
Wnt-luc
30000
20000
10000
0
Control
Wnt3a
Wnt3a + Dkk1
Wnt3a + Dkk1 + 16A12 (1)
Wnt3a + Dkk1 + 16A12 (3)
Wnt3a + Dkk1 + 16A12 (10)
Wnt3a + Dkk1 + 16A12 (30)
Wnt3a + Dkk1 + 16A12 (100)
Wnt3a + Dkk1 + 16A12 (300)
Wnt3a + Dkk1 + 16A12p (1)
Wnt3a + Dkk1 + 16A12p (3)
Wnt3a + Dkk1 + 16A12p (10)
Wnt3a + Dkk1 + 16A12p (30)
Wnt3a + Dkk1 + 16A12p (100)
Wnt3a + Dkk1 + 16A12p (300)
Wnt3a + Dkk1 + 18B5 (1)
Wnt3a + Dkk1 + 18B5 (3)
Wnt3a + Dkk1 + 18B5 (10)
Wnt3a + Dkk1 + 18B5 (30)
Wnt3a + Dkk1 + 18B5 (100)
Wnt3a + Dkk1 + 18B5 (300)
Wnt3a + Dkk1 + 18B5p (1)
Wnt3a + Dkk1 + 18B5p (3)
Wnt3a + Dkk1 + 18B5p (10)
Wnt3a + Dkk1 + 18B5p (30)
Wnt3a + Dkk1 + 18B5p (100)
Wnt3a + Dkk1 + 18B5p (300)
(nM)
p = extra purified
*Significant vs. control, p<0.05
rcWnt3a (10 ng/ml)
rchDkk1 (300 ng/ml)

Figure 3B


Figure 3C
Wnt activity in KS483 Wnt reporter cell line
Wnt-luc
40000
30000
20000
10000
0
Control
Wnt3a
Wnt3a + rcmDkk1
Wnt3a + rcmDkk1 + 4E4p
Wnt3a + rcmDkk1 + 10A5p
Wnt3a + rcmDkk1 + 16A12p
Wnt3a + rcmDkk1 + 18B5p
Wnt3a + rcmDkk1 + 21C12p
p = extra purified
*Significant vs. control, p<0.05
rcWnt3a (10 ng/ml)
rcmDkk1 (500 ng/ml)
Nanobodies (1000 nM)


Figure 4A
ALP activity in KS483 cells
ALP activity
100
75
50
25
0
Control
BMP2
BMP2 + DKK1
BMP2 + DKK1 + 4E4p (3)
BMP2 + DKK1 + 4E4p (10)
BMP2 + DKK1 + 4E4p (30)
BMP2 + DKK1 + 4E4p (100)
BMP2 + DKK1 + 4E4p (300)
BMP2 + DKK1 + 10A5p (3)
BMP2 + DKK1 + 10A5p (10)
BMP2 + DKK1 + 10A5p (30)
BMP2 + DKK1 + 10A5p (100)
BMP2 + DKK1 + 10A5p (300)
BMP2 + DKK1 + 16A12p (3)
BMP2 + DKK1 + 16A12p (10)
BMP2 + DKK1 + 16A12p (30)
BMP2 + DKK1 + 16A12p (100)
BMP2 + DKK1 + 16A12p (300)
BMP2 + DKK1 + 18B5p (3)
BMP2 + DKK1 + 18B5p (10)
BMP2 + DKK1 + 18B5p (30)
BMP2 + DKK1 + 18B5p (100)
BMP2 + DKK1 + 18B5p (300)
BMP2 + DKK1 + 21C12p (3)
BMP2 + DKK1 + 21C12p (10)
BMP2 + DKK1 + 21C12p (30)
BMP2 + DKK1 + 21C12p (100)
BMP2 + DKK1 + 21C12p (300)
(nM)
p = extra purified
*Significant vs. control, p<0.05
rcBMP2 (100 ng/ml)
rchDkk1 (1000 ng/ml)

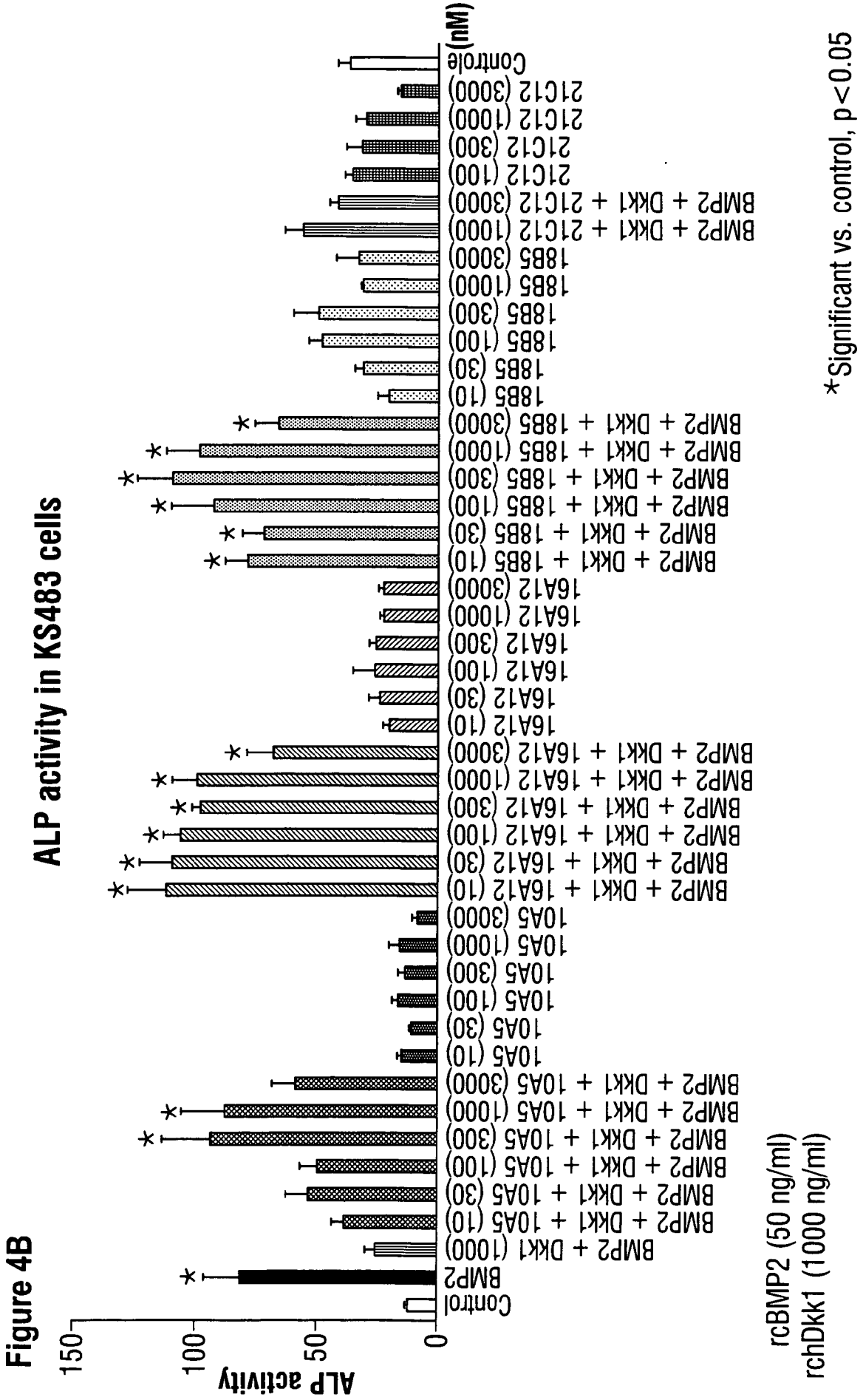
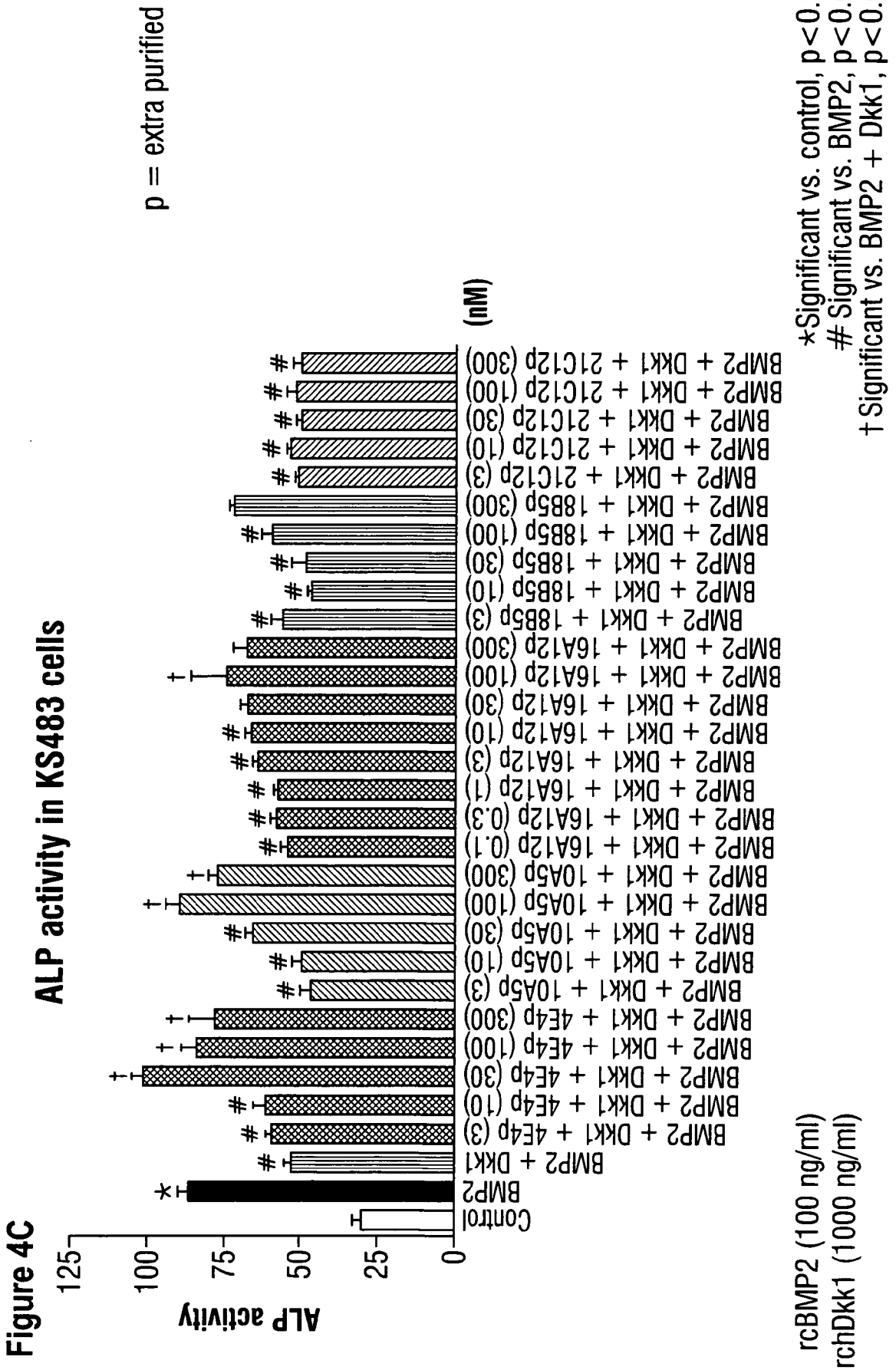

ALP activity in KS483 cells
ALP activity
150
100
50
0
Control
BMP2
BMP2 + Dkk1 (1000)
BMP2 + Dkk1 + 10A5 (10)
BMP2 + Dkk1 + 10A5 (30)
BMP2 + Dkk1 + 10A5 (100)
BMP2 + Dkk1 + 10A5 (300)
BMP2 + Dkk1 + 10A5 (1000)
BMP2 + Dkk1 + 10A5 (3000)
10A5 (10)
10A5 (30)
10A5 (100)
10A5 (300)
10A5 (1000)
10A5 (3000)
BMP2 + Dkk1 + 16A12 (10)
BMP2 + Dkk1 + 16A12 (30)
BMP2 + Dkk1 + 16A12 (100)
BMP2 + Dkk1 + 16A12 (300)
BMP2 + Dkk1 + 16A12 (1000)
BMP2 + Dkk1 + 16A12 (3000)
16A12 (10)
16A12 (30)
16A12 (100)
16A12 (300)
16A12 (1000)
16A12 (3000)
BMP2 + Dkk1 + 18B5 (10)
BMP2 + Dkk1 + 18B5 (30)
BMP2 + Dkk1 + 18B5 (100)
BMP2 + Dkk1 + 18B5 (300)
BMP2 + Dkk1 + 18B5 (1000)
BMP2 + Dkk1 + 18B5 (3000)
18B5 (10)
18B5 (30)
18B5 (100)
18B5 (300)
18B5 (1000)
18B5 (3000)
BMP2 + Dkk1 + 21C12 (1000)
BMP2 + Dkk1 + 21C12 (3000)
21C12 (100)
21C12 (300)
21C12 (1000)
21C12 (3000)
Controle
(nM)
*Significant vs. control, p<0.05
rcBMP2 (50 ng/ml)
rchDkk1 (1000 ng/ml)

Figure 4B


Figure 4C
ALP activity in KS483 cells
ALP activity
125
100
75
50
25
0
Control
BMP2
BMP2 + DKK1
BMP2 + DKK1 + 4E4p (3)
BMP2 + DKK1 + 4E4p (10)
BMP2 + DKK1 + 4E4p (30)
BMP2 + DKK1 + 4E4p (100)
BMP2 + DKK1 + 4E4p (300)
BMP2 + DKK1 + 10A5p (3)
BMP2 + DKK1 + 10A5p (10)
BMP2 + DKK1 + 10A5p (30)
BMP2 + DKK1 + 10A5p (100)
BMP2 + DKK1 + 10A5p (300)
BMP2 + DKK1 + 16A12p (0.1)
BMP2 + DKK1 + 16A12p (0.3)
BMP2 + DKK1 + 16A12p (1)
BMP2 + DKK1 + 16A12p (3)
BMP2 + DKK1 + 16A12p (10)
BMP2 + DKK1 + 16A12p (30)
BMP2 + DKK1 + 16A12p (100)
BMP2 + DKK1 + 16A12p (300)
BMP2 + DKK1 + 18B5p (3)
BMP2 + DKK1 + 18B5p (10)
BMP2 + DKK1 + 18B5p (30)
BMP2 + DKK1 + 18B5p (100)
BMP2 + DKK1 + 18B5p (300)
BMP2 + DKK1 + 21C12p (3)
BMP2 + DKK1 + 21C12p (10)
BMP2 + DKK1 + 21C12p (30)
BMP2 + DKK1 + 21C12p (100)
BMP2 + DKK1 + 21C12p (300)
(nM)
p = extra purified
*Significant vs. control, p<0.05
Significant vs. BMP2, p<0.05
† Significant vs. BMP2 + Dkk1, p<0.05
rcBMP2 (100 ng/ml)
rchDkk1 (1000 ng/ml)

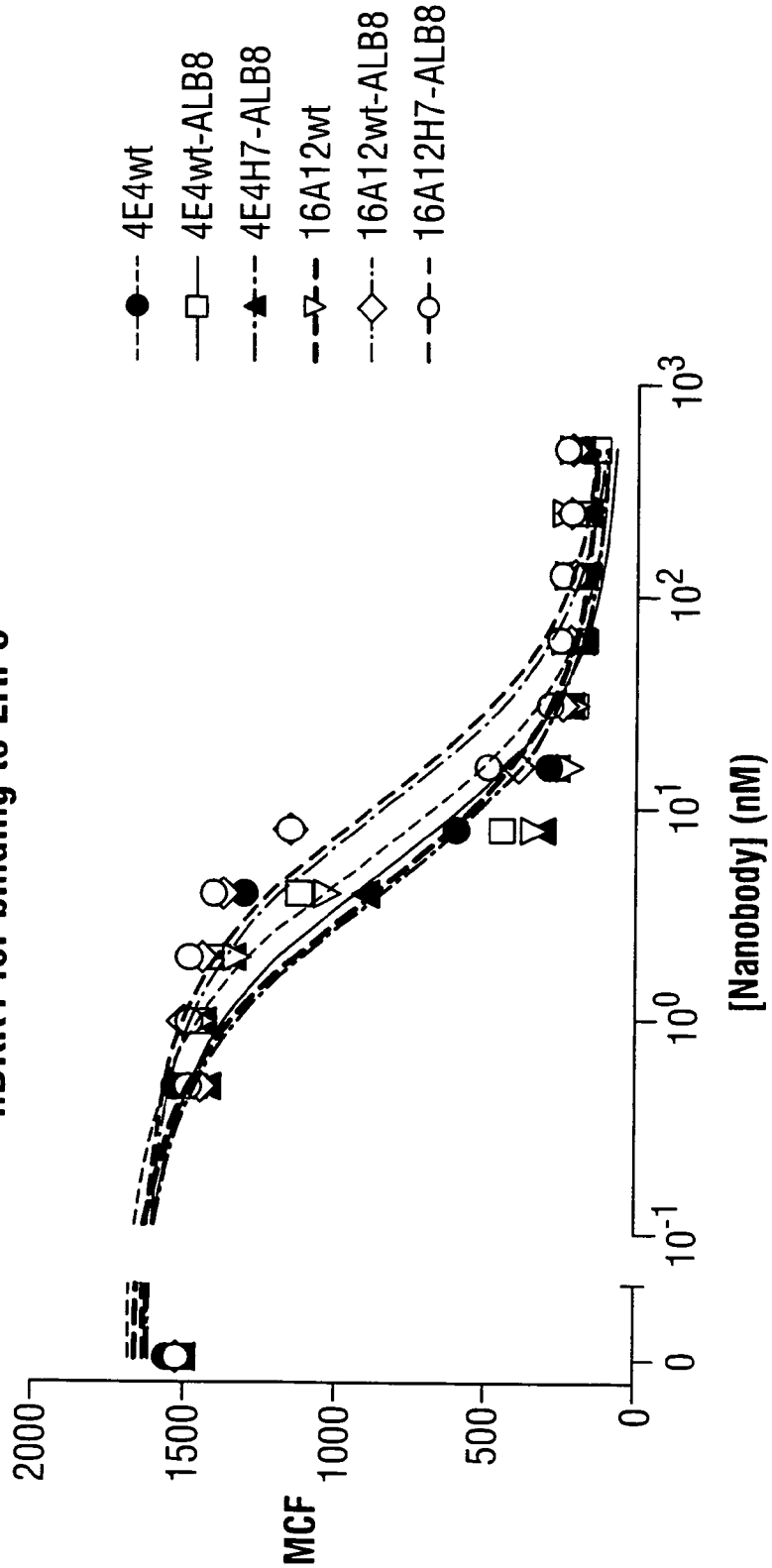
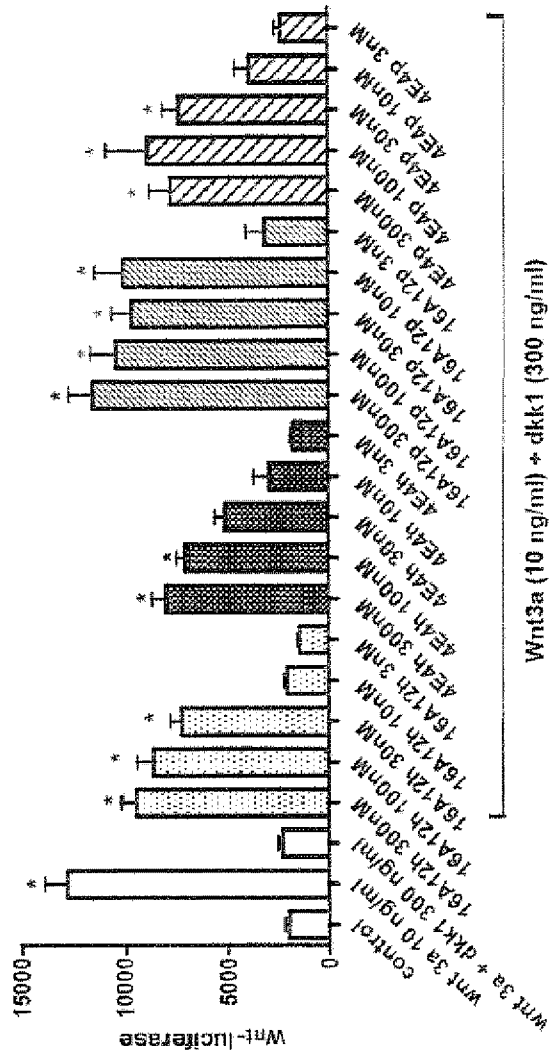

Competition of anti-DKK1 Nanobodies with hDKK1 for binding to LRP5
4E4wt
4E4wt-ALB8
4E4H7-ALB8
16A12wt
16A12wt-ALB8
16A12H7-ALB8
MCF
2000
1500
1000
500
0
0
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
[Nanobody] (nM)

Figure 5

Figure 6

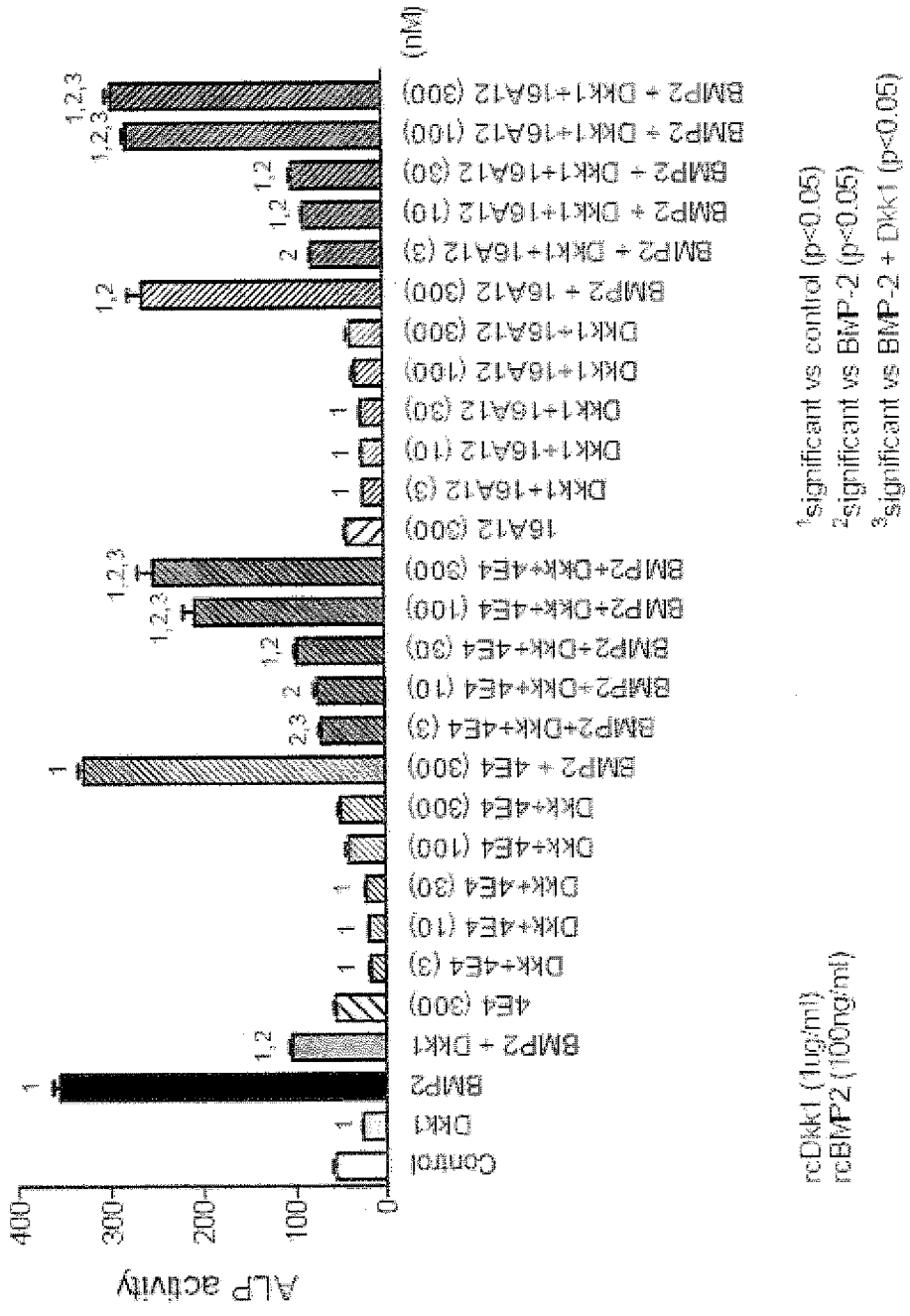


* Significant vs control ; p<0.05

h = humanized, bispecific Nanobodies

p = wild-type (highly purified) monovalent Nanobodies

Figure 7


ALP activity in KS483 cells
ALP activity
400
300
200
100
0
Control
DKK1
BMP2
BMP2 + DKK1
4E4 (300)
DKK+4E4 (3)
DKK+4E4 (10)
DKK+4E4 (30)
DKK+4E4 (100)
DKK+4E4 (300)
BMP2 + 4E4 (300)
BMP2+DKK+4E4 (3)
BMP2+DKK+4E4 (10)
BMP2+DKK+4E4 (30)
BMP2+DKK+4E4 (100)
BMP2+DKK+4E4 (300)
16A12 (300)
DKK1+16A12 (3)
DKK1+16A12 (10)
DKK1+16A12 (30)
DKK1+16A12 (100)
DKK1+16A12 (300)
BMP2 + 16A12 (300)
BMP2 + DKK1+16A12 (3)
BMP2 + DKK1+16A12 (10)
BMP2 + DKK1+16A12 (30)
BMP2 + DKK1+16A12 (100)
BMP2 + DKK1+16A12 (300)
(nM)
rcDkk1 (1ug/ml)
rcBMP2 (100ng/ml)
1significant vs control (p<0.05)
2significant vs BMP-2 (p<0.05)
3significant vs BMP-2 + Dkk1 (p<0.05)

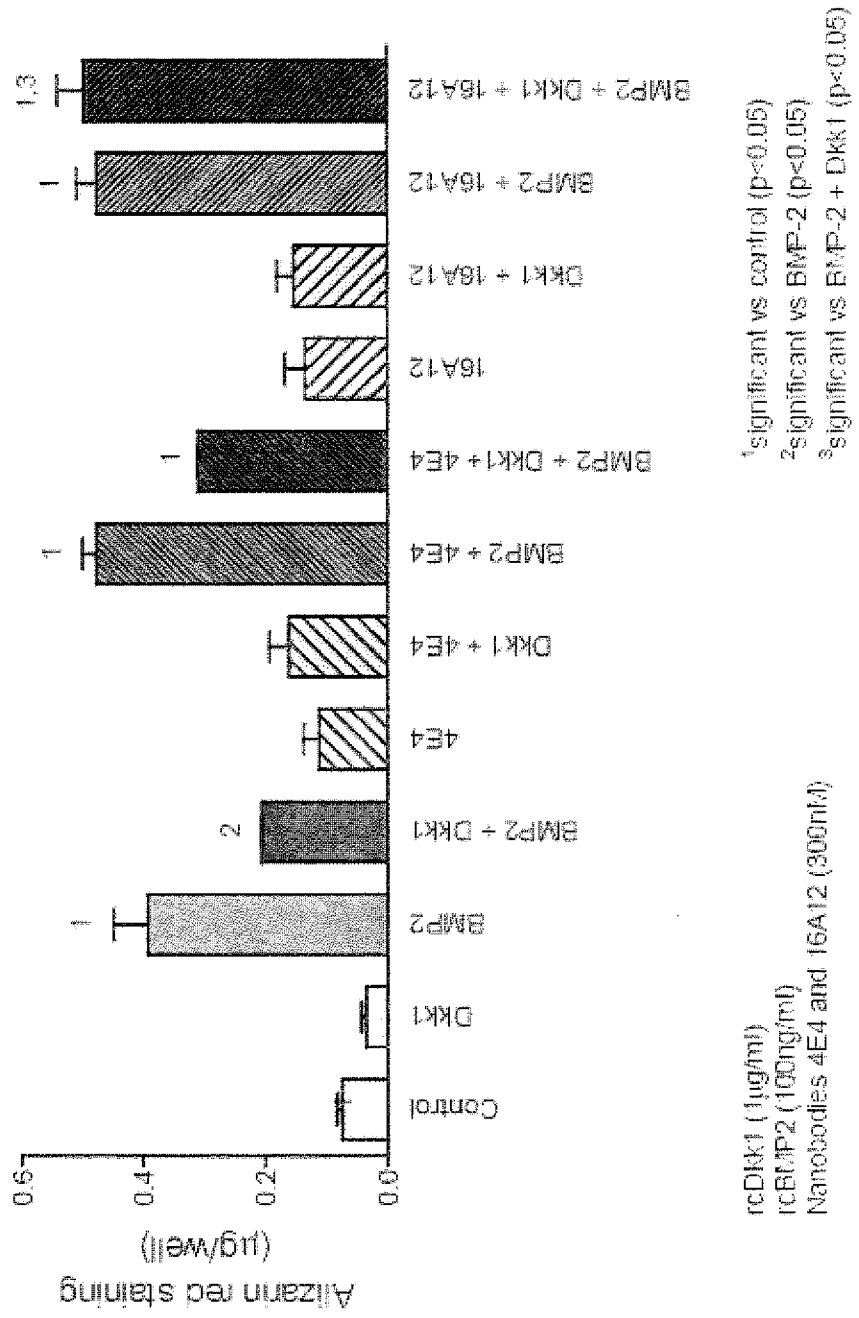

Mineralization in KS483 cells
Alizarin red staining (µg/well)
0.0
0.2
0.4
0.6
Control
DKK1
BMP2
BMP2 + DKK1
4E4
DKK1 + 4E4
BMP2 + 4E4
BMP2 + DKK1 + 4E4
16A12
DKK1 + 16A12
BMP2 + 16A12
BMP2 + DKK1 + 16A12
1
1
2
1
1
1
1
1,3
rcDKK1 (1µg/ml)
rcBMP2 (100ng/ml)
Nanobodies 4E4 and 16A12 (300nM)
1significant vs control (p<0.05)
2significant vs BMP-2 (p<0.05)
3significant vs BMP-2 + DKK1 (p<0.05)

Figure 8

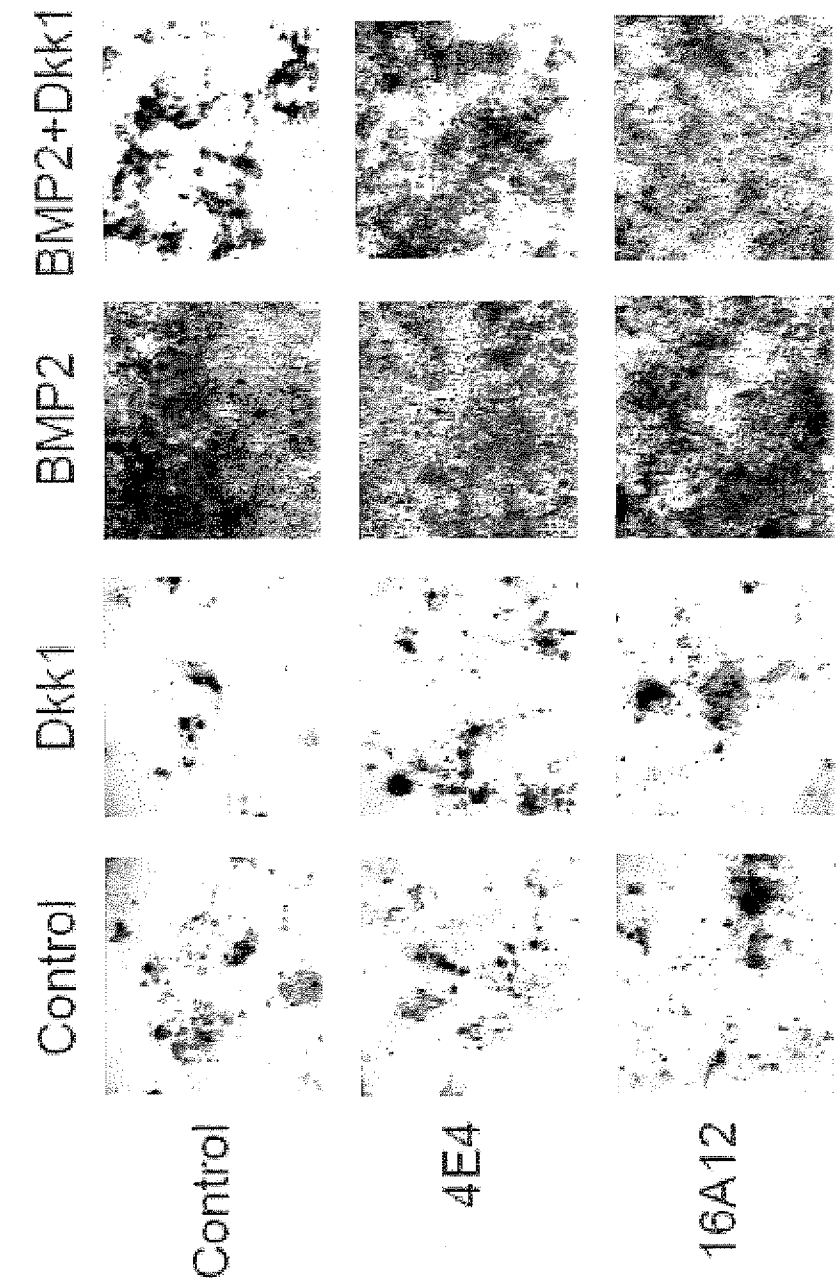


Figure 9