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(54) Title: PD1-4-1BBL VARIANT FUSION PROTEIN AND METHODS OF USE THEREOF

(57) **Abrégé/Abstract:**

PD1-4-1BBL variant fusion proteins are provided. Also provided are isolated polypeptides comprising a PD1 variant or a 4-1BBL variant. Also provided are polynucleotides and nucleic acid constructs encoding the PD1-4-1BBL fusion protein or the isolated polypeptide, host-cells expressing the same and methods of use thereof.

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(54) Title: PD1-4-IBBL VARIANT FUSION PROTEIN AND METHODS OF USE THEREOF

(57) Abstract: PD1-4-IBBL variant fusion proteins are provided. Also provided are isolated polypeptides comprising a PD1 variant or a 4-IBBL variant. Also provided are polynucleotides and nucleic acid constructs encoding the PD1-4-IBBL fusion protein or the isolated polypeptide, host-cells expressing the same and methods of use thereof.

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CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 167

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
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PD1-4-1BBL VARIANT FUSION PROTEIN AND METHODS OF USE THEREOF

RELATED APPLICATION

This application claims the benefit of priority under 35 USC §119(e) of U.S. Provisional Patent Application No. 62/696,365 filed July 11, 2018 and U.S. Provisional Patent Application No. 62/786,599 filed on December 31, 2018, the contents of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING STATEMENT

The ASCII file, entitled 76050SequenceListing.txt, created on 7 July 2019, comprising 268 kilobytes, submitted concurrently with the filing of this application is incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to a PD1-4-1BBL variant fusion protein and methods of use thereof.

Dual Signaling Proteins (DSP), also known as Signal-Converting-Proteins (SCP), which are currently known in the art as bi-functional fusion proteins that link an extracellular portion of a type I membrane protein (extracellular amino-terminus), to an extracellular portion of a type II membrane protein (extracellular carboxyl-terminus), forming a fusion protein with two active sides (see e.g. US Patent Nos. 7,569,663 and 8,039,437).

PD1 is a surface co-inhibitory receptor of the immunoglobulin super family. PD1 is expressed on T cells, B cells, monocytes, natural killer cells, dendritic cells and many tumor-infiltrating lymphocytes (TILs). PD1 has two ligands: PDL1 (also named B7H1; CD274) and PDL2 (B7DC; CD273), that are both co-inhibitory. PDL1 is expressed on resting and activated T cells, B cells, dendritic cells, macrophage, vascular endothelial cells and pancreatic islet cells. PDL1 is also known to be expressed in various types of cancers, especially in NSCLC, melanoma, renal cell carcinoma, gastric cancer, hepatocellular as well as cutaneous and various leukemia cancers, multiple myeloma and others. PDL2 expression is seen on macrophages and dendritic cells alone and is far less prevalent than PDL1 across tumor types. The expression of PDL1 is induced by multiple proinflammatory molecules, including types I and II IFN- γ , TNF- α , LPS, GM-CSF and VEGF, as well as the cytokines IL-10 and IL-4, with IFN- γ being the most potent inducer. The tumor micro-environment upregulates PDL1 expression, thereby, promoting immune suppression. In response to immune attack, cancer cells overexpress PDL1, which binds to PD1 receptor on T

cells, inhibiting the activation of T-cells, thus suppressing T-cell attack and inducing tumor immune escape. PD1/PDL1 pathway regulates immune suppression by several mechanisms:

- Induces apoptosis of activated T cells;
- Restrains cytotoxic T lymphocytes (CTL-CD8) activity;
- 5 • Inhibits the proliferation of T cells;
- Facilitates T cell anergy and exhaustion;
- Enhances the function of regulatory T cells; and
- Restrains impaired T cell activation and IL-2 production.

4-1BBL is the activating ligand of the 4-1BB receptor (CD137), a member of the TNF receptor
 10 superfamily and a potent activation-induced T cell costimulatory molecule. 4-1BBL naturally forms a homo-trimer but signaling via 4-1BB requires significant oligomerization of 4-1BBL. 4-1BBL is present on a variety of antigen presenting cells (APCs), including dendritic cells (DCs), B cells, and macrophages. The 4-1BB receptor is not detected (<3 %) on resting T cells or T cell lines; however, 4-1BB is stably upregulated when T cells are activated. 4-1BB activation
 15 upregulates survival genes, enhances cell division, induces cytokine production and prevents activation induced cell death in T-cells.

Additional background art includes:

Maute et al. PNAS, 2015 Nov 24;112(47):E6506-14;

International Patent Application Publication No. WO 2018053885;

20 International Patent Application Publication No. WO 2018032793;

International Patent Application Publication No. WO 2018006881;

International Patent Application Publication No. WO 2013144704;

International Patent Application Publication No. WO 2016022994;

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25 International Patent Application Publication No. WO 2018085358;

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International Patent Application Publication No. WO 2014180288;

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30 International Patent Application Publication No. WO2001/049318;

International Patent Application Publication No. WO2016/139668;

International Patent Application Publication No. WO2014/106839;

International Patent Application Publication No. WO2012/042480;

US Patent Application Publication No. 20150183881;

US Patent Application Publication No. US20070110746;

US Patent Application Publication No. US20070036783; and

US Patent No. US9562087.

5 SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL amino acid sequence, wherein the PD1 amino acid sequence:

10 (a) is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or is 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and does not comprise an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and/or

15 (b) comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and/or

wherein the 4-1BBL amino acid sequence:

(aa) is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and does not comprise an amino acid segment G198-E205 corresponding to SEQ ID NO: 3, is 170 – 182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and does not comprise an amino acid segment A1-E23 corresponding to SEQ ID NO: 3 or is 184 amino acids in length having at least 80 % identity to SEQ ID NO: 123; and/or

25 (bb) comprises three repeats of a 4-1BBL amino acid sequence;

and wherein the fusion protein is capable of at least one of:

(i) binding PDL1 and 4-1BB;

(ii) activating the 4-1BB signaling pathway in a cell expressing the 4-1BB; and/or

(iii) co-stimulating immune cells expressing the 4-1BB.

30 According to an aspect of some embodiments of the present invention there is provided a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL amino acid sequence, wherein the PD1 amino acid sequence:

(a) is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93 and does not

4

comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2 and/or

(b) comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and/or

5 wherein the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25; and wherein the fusion protein is capable of at least one of:

(i) binding PDL1 and 4-1BB;

(ii) activating the 4-1BB signaling pathway in a cell expressing the 4-1BB; and/or

10 (iii) co-stimulating immune cells expressing the 4-1BB.

According to an aspect of some embodiments of the present invention there is provided a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL amino acid sequence, wherein the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21
15 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and/or wherein the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25; and wherein the fusion protein is capable of at least one of:

(i) binding PDL1 and 4-1BB;

20 (ii) activating the 4-1BB signaling pathway in a cell expressing the 4-1BB; and/or

(iii) co-stimulating immune cells expressing the 4-1BB.

According to an aspect of some embodiments of the present invention there is provided a PD1-4-1BBL fusion protein comprising a $(GGGGS)_n$ ($n=1-4$) linker between the PD1 and the 4-1BBL, wherein the fusion protein is capable of at least one of:

25 (i) binding PDL1 and 4-1BB;

(ii) activating the 4-1BB signaling pathway in a cell expressing the 4-1BB; and/or

(iii) co-stimulating immune cells expressing the 4-1BB.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to SEQ ID NO: 145.

30 According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 145.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 145.

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According to an aspect of some embodiments of the present invention there is provided an isolated polypeptide comprising a PD1 amino acid sequence, wherein the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and does not comprise
5 any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or is 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and does not comprise an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and optionally comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and wherein the
10 polypeptide is capable of binding PDL1.

According to an aspect of some embodiments of the present invention there is provided an isolated polypeptide comprising a PD1 amino acid sequence, wherein the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93 and does not comprise
15 any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and optionally comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and wherein the polypeptide is capable of binding PDL1.

According to an aspect of some embodiments of the present invention there is provided an isolated polypeptide comprising a PD1 amino acid sequence, wherein the PD1 amino acid
20 sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and wherein the polypeptide is capable of binding PDL1.

According to an aspect of some embodiments of the present invention there is provided an
25 isolated polypeptide comprising a 4-1BBL amino acid sequence, wherein the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and does not comprise an amino acid segment G198-E205 corresponding to
30 SEQ ID NO: 3, or is 170 – 182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and does not comprise an amino acid segment A1-E23 corresponding to SEQ ID NO: 3; and optionally comprises three repeats of the 4-1BBL amino acid sequence; and wherein the polypeptide is capable of at least one of:

- (i) binding 4-1BB,

- (ii) activating the 4-1BB signaling pathway in a cell expressing the 4-1BB; and/or
- (iii) co-stimulating immune cells expressing the 4-1BB.

According to an aspect of some embodiments of the present invention there is provided an isolated polypeptide comprising a 4-1BBL amino acid sequence, wherein the 4-1BBL amino acid
 5 sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25; and wherein the polypeptide is capable of at least one of:

- (i) binding 4-1BB,
- (ii) activating the 4-1BB signaling pathway in a cell expressing the 4-1BB; and/or
- 10 (iii) co-stimulating immune cells expressing the 4-1BB.

According to some embodiments of the invention, the PD1 amino acid sequence is no more than 140 amino acids long.

According to some embodiments of the invention, the PD1 amino acid sequence is at least 126 amino acids long.

15 According to some embodiments of the invention, the PD1 amino acid sequence comprises the SEQ ID NO: 18, 19, 20 or 21.

According to some embodiments of the invention, the PD1 amino acid sequence comprises the SEQ ID NO: 18, 19, 20, 21, 85, 89 or 93.

20 According to some embodiments of the invention, the PD1 amino acid sequence comprises the SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 or 115.

According to some embodiments of the invention, the PD1 amino acid sequence comprises the SEQ ID NO: 117 or 119.

According to some embodiments of the invention, the PD1 amino acid sequence comprises SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95.

25 According to some embodiments of the invention, the PD1 amino acid sequence comprises SEQ ID NO: 75, 83, 87, 91 or 95.

According to some embodiments of the invention, the PD1 amino acid sequence comprises SEQ ID NO: 73.

30 According to some embodiments of the invention, the PD1 amino acid sequence comprises the SEQ ID NO: 18.

According to some embodiments of the invention, the PD1 amino acid sequence consists of the SEQ ID NO: 18, 19, 20 or 21.

According to some embodiments of the invention, the PD1 amino acid sequence consists of the SEQ ID NO: 18, 19, 20, 21, 85, 89 or 93.

According to some embodiments of the invention, the PD1 amino acid sequence consists of the SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 or 115.

According to some embodiments of the invention, the PD1 amino acid sequence consists of the SEQ ID NO: 117 or 119.

5 According to some embodiments of the invention, the PD1 amino acid sequence consists of SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95.

According to some embodiments of the invention, the PD1 amino acid sequence consists of SEQ ID NO: 75, 83, 87, 91 or 95.

10 According to some embodiments of the invention, the PD1 amino acid sequence consists of SEQ ID NO: 73.

According to some embodiments of the invention, the PD1 amino acid sequence consists of the SEQ ID NO: 18.

15 According to some embodiments of the invention, the 4-1BBL amino acid sequence has at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 22-24.

According to some embodiments of the invention, the 4-1BBL amino acid sequence does not comprise any of amino acid residues A1 – V6 or A1 – G14 corresponding to SEQ ID NO: 3.

According to some embodiments of the invention, the 4-1BBL amino acid sequence comprises the SEQ ID NO: 22, 23, 24 or 25.

20 According to some embodiments of the invention, the 4-1BBL amino acid sequence consists of the SEQ ID NO: 22, 23, 24 or 25.

According to some embodiments of the invention, the 4-1BBL amino acid sequence comprises the SEQ ID NO: 125, 127 or 129.

25 According to some embodiments of the invention, the 4-1BBL amino acid sequence consists of the SEQ ID NO: 125, 127 or 129.

According to some embodiments of the invention, the 4-1BBL amino acid sequence comprises the SEQ ID NO: 123.

According to some embodiments of the invention, the 4-1BBL amino acid sequence consists of the SEQ ID NO: 123.

30 According to some embodiments of the invention, the PD1-4-1BBL fusion protein comprises a linker between the PD1 and the 4-1BBL.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein or the isolated polypeptide comprising a linker between each of the three repeats of the 4-1BBL amino acid sequence.

According to some embodiments of the invention, the linker has a length of one to six amino acids.

According to some embodiments of the invention, the linker is a single amino acid linker.

According to some embodiments of the invention, the linker is glycine.

5 According to some embodiments of the invention, the linker is not an Fc domain of an antibody or a fragment thereof.

According to some embodiments of the invention, the linker is an Fc domain of an antibody or a fragment thereof.

10 According to some embodiments of the invention, the linker is a (GGGGS)_n(n=1-4) (SEQ ID NO: 149) linker.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein being in a form of at least a homo-trimer.

According to some embodiments of the invention, the at least homo-trimer is at least 100 kD in molecular weight as determined by SEC-MALS.

15 According to some embodiments of the invention, the PD1-4-1BBL fusion protein or the isolated polypeptide being soluble.

According to some embodiments of the invention, the production yield of the fusion protein is at least 1.5 fold higher than the production yield of SEQ ID NO: 5 under the same production conditions, the production conditions comprise expression in a mammalian cell and culturing at
20 32 – 37 °C, 5 – 10 % CO₂ for 5 - 13 days.

According to some embodiments of the invention, the amount of aggregates of the fusion protein is at least 20 % lower than the amount of aggregates of SEQ ID NO: 5 under the same production conditions, the aggregates are of at least 300 kDa in molecular weight and the production conditions comprise expression in a mammalian cell and culturing at 32 – 37 °C, 5 –
25 10 % CO₂ for 5 - 13 days.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147.

30 According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid

sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107 and 111.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid
5 sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid
10 sequence comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16 and 44-56.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16.

According to some embodiments of the invention, the at least 80 % identity comprises at least
15 90 % identity.

According to some embodiments of the invention, the at least 80 % identity comprises at least 95 % identity.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid
20 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid
25 sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107 and 111.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO:
30 97, 101, 103, 105, 107 and 111.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16 and 44-56.

5 According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147.

10 According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 15 12, 14, 16, 44-56, 97, 101, 103, 105, 107 and 111.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111.

20 According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107.

According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence consist of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16 and 44-56.

25 According to some embodiments of the invention, the PD1-4-1BBL fusion protein amino acid sequence consist of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16.

According to an aspect of some embodiments of the present invention there is provided a polynucleotide encoding the PD1-4-1BBL fusion protein or the isolated polypeptide.

30 According to an aspect of some embodiments of the present invention there is provided a nucleic acid construct comprising the polynucleotide, and a regulatory element for directing expression of the polynucleotide in a host cell.

According to some embodiments of the invention, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72, 98, 102, 104, 106, 108, 112, 114, 134, 136, 138, 140, 142, 144 and 148.

5 According to some embodiments of the invention, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72, 98, 102, 104, 106, 108 and 112.

According to some embodiments of the invention, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 98, 102, 104, 106, 108 and 112.

10 According to some embodiments of the invention, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 98, 102, 104, 106 and 108.

According to some embodiments of the invention, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72.

According to some embodiments of the invention, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 70 – 72.

15 According to some embodiments of the invention, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 134, 136, 138, 140, 142, 144 and 148.

According to some embodiments of the invention, the polynucleotide comprises SEQ ID NO: 146.

20 According to an aspect of some embodiments of the present invention there is provided a host cell comprising the PD1-4-1BBL fusion protein or the polypeptide or the polynucleotide or the nucleic acid construct.

According to an aspect of some embodiments of the present invention there is provided a method of producing a PD1-4-1BBL fusion protein or a polypeptide, the method comprising expressing in a host cell the polynucleotide or the nucleic acid construct.

25 According to some embodiments of the invention, the method comprising isolating the fusion protein or the polypeptide.

30 According to an aspect of some embodiments of the present invention there is provided a method of treating a disease that can benefit from activating immune cells comprising administering to a subject in need thereof the PD1-4-1BBL fusion protein or the isolated polypeptide, the polynucleotide or the nucleic acid construct or the host cell.

According to some embodiments of the invention, the method further comprising administering to the subject a therapeutic agent for treating the disease.

According to an aspect of some embodiments of the present invention there is provided the PD1-4-1BBL fusion protein or the isolated polypeptide, the polynucleotide or the nucleic acid

construct or the host cell, for use in treating a disease that can benefit from activating immune cells.

According to some embodiments of the invention, the composition further comprising a therapeutic agent for treating the disease.

5 According to an aspect of some embodiments of the present invention there is provided an article of manufacture comprising a packaging material packaging a therapeutic agent for treating a disease that can benefit from activating immune cells; and the PD1-4-1BBL fusion protein or the isolated polypeptide, the polynucleotide or the nucleic acid construct or the host cell.

According to some embodiments of the invention, cells of the disease express PDL1.

10 According to some embodiments of the invention, the disease comprises a hyper-proliferative disease.

According to some embodiments of the invention, the hyper-proliferative disease comprises sclerosis, fibrosis, Idiopathic pulmonary fibrosis, psoriasis, systemic sclerosis/scleroderma, primary biliary cholangitis, primary sclerosing cholangitis, liver fibrosis, prevention of radiation-
15 induced pulmonary fibrosis, myelofibrosis or retroperitoneal fibrosis.

According to some embodiments of the invention, the hyper-proliferative disease comprises cancer.

According to some embodiments of the invention, the cancer is selected from the group consisting of lymphoma, leukemia and carcinoma.

20 According to some embodiments of the invention, the disease comprises a disease associated with immune suppression or medication induced immunosuppression.

According to some embodiments of the invention, the disease comprises HIV, Measles, influenza, LCCM, RSV, Human Rhinoviruses, EBV, CMV or Parvo viruses.

According to some embodiments of the invention, the disease comprises an infection.

25 According to an aspect of some embodiments of the present invention there is provided a method of activating immune cells, the method comprising in-vitro activating immune cells in the presence of the PD1-4-1BBL fusion protein or the isolated polypeptide, the polynucleotide or the nucleic acid construct or the host cell.

30 According to some embodiments of the invention, the activating is in the presence of cells expressing PDL1 or exogenous PDL1.

According to some embodiments of the invention, the cells expressing the PDL1 comprise pathologic cells.

According to some embodiments of the invention, the pathologic cells comprise cancer cells.

According to some embodiments of the invention, the activating is in the presence of an anti-cancer agent.

According to some embodiments of the invention, the therapeutic agent for treating the disease or the anti-cancer agent comprises an antibody.

5 According to some embodiments of the invention, the antibody is selected from the group consisting rituximab, cetuximab, trastuzumab, edrecolomab, almetuzumab, gemtuzumab, ibritumomab, panitumumab, Belimumab, Bevacizumab, Bivatuzumab mertansine, Blinatumomab, Blontuvetmab, Brentuximab vedotin, Catumaxomab, Cixutumumab, Daclizumab, Adalimumab, Bezlotoxumab, Certolizumab pegol, Citatuzumab bogatox, Daratumumab, 10 Dinutuximab, Elotuzumab, Ertumaxomab, Etaracizumab, Gemtuzumab ozogamicin, Girentuximab, Necitumumab, Obinutuzumab, Ofatumumab, Pertuzumab, Ramucirumab, Siltuximab, Tositumomab, Trastuzumab and ipilimumab.

According to some embodiments of the invention, the antibody is selected from the group consisting of rituximab and cetuximab.

15 According to some embodiments of the invention, the therapeutic agent for treating the disease or the anti-cancer agent comprises an IMiD (e.g. Thalidomide, Lenalidomide, Pomalidomide).

According to some embodiments of the invention, the method comprising adoptively transferring the immune cells following the activating to a subject in need thereof.

According to some embodiments of the invention, the immune cells comprise T cells.

20 Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In 25 addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

30 Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIG. 1 is a schematic representation of the PD1-4-1BBL fusion protein referred to herein as “DSP105” (SEQ ID NO: 5) comprising an N-terminal signal peptide and a His-tag (SEQ ID NO: 26). Shown the signal peptide (black underlined, SEQ ID NO: 4), the N-terminal His-tag (black bold), the PD1 domain (red, SEQ ID NO: 2), the glycine linker (black) and the 4-1BBL domain (blue, SEQ ID NO: 3) and

FIGs. 2A-D demonstrate the predicted 3D structure of DSP105 (SEQ ID NO: 5). Figure 2A is a schematic 3D model. PD1 is shown in grey ribbons, PDL1 (PD1 ligand) is shown in green ribbons, 4-1BBL is shown in blue ribbons and 2 additional copies of 4-1BBL (forming the trimer) are shown in light blue. Figure 2B is a schematic, full atomic 3D model. Figures 2C-D are schematic 3D models. The X-ray resolved domains are represented by its surface and colored by a hydrophobicity scale - blue (most hydrophilic) to Brown (Hydrophobic).

FIG. 3 is a schematic representation of the domain and segments identified in DSP105 (SEQ ID NO: 5). An Ig-like V-type domain is highlighted in light blue (the underlined sequence was resolved in X-ray), An X-ray resolved part is highlighted in grey and flanking/unstructured regions are marked with red boxes.

FIG. 4 demonstrates the predicted 3D structure of PD1-4-1BBL variant fusion protein referred to herein as “DSP105_var1” (SEQ ID NO: 12). In the upper panel, the X-ray resolved domains are displayed in surface representation colored by hydrophobicity scale - Blue (most hydrophilic) to Brown (Hydrophobic). PDL1 (PD1 ligand) is presented in green ribbons. In the lower panel, 4-1BBL is presented in red ribbons, and PD1 in blue ribbons.

FIG. 5 demonstrates the predicted 3D structure of PD1-4-1BBL variant fusion protein referred to herein as “DSP105_var2” (SEQ ID NO: 14). The color code is the same as in Figure 4.

FIG. 6 demonstrates the predicted 3D structure of PD1-4-1BBL variant fusion protein referred to herein as “DSP105_var3” (SEQ ID NO: 16). The color code is the same as in Figure 4.

FIG. 7 presents images of SDS-PAGE analysis of N-terminal His-tagged DSP105 (SEQ ID NO: 1), expressed in Expi293 cells; and N-terminal His-tagged DSP105_var17 (SEQ ID NO: 99), expressed in CHO-3E7. Both fusion proteins were purified by a single step affinity purification on a Nickel column and separated under reducing and non-reducing conditions, as indicated. The samples presented in the images are as follows: Lane M - molecular weight marker; Lanes 1-2: Two separate preparations of N-terminal His-tagged DSP105 (SEQ ID NO: 1), Lane 3 - N-terminal His-tagged DSP105_var17 (SEQ ID NO: 99).

FIGs. 8A-E present images of SDS-PAGE analysis (marked as PAGE) and western blot (marked as WB) analysis using the indicated antibody of the PD1-4-1BBL variant fusion proteins

produced in CHO-3E7 cells. Shown are PD1-4-1BBL variant fusion proteins referred to herein as “N-terminal His-tagged DSP105_var17” (SEQ ID NO: 99, Figure 8A), “DSP105_var18” (SEQ ID NO: 101, Figure 8B), “DSP105_var19” (SEQ ID NO: 103, Figure 8C), “DSP105_var20” (SEQ ID NO: 105, Figure 8D) and “DSP105_var23” (SEQ ID NO: 113, Figure 8E) under reducing or non-reducing conditions. The samples presented in the images are as follows: Lane M - molecular weight marker; Lanes 1 to 3 - cell culture supernatants collected on day 2, day 4 and day 5 post-transfection, respectively, under reducing conditions; Lane NC1 - negative control under reducing conditions; Lanes 4 to 6 - cell culture supernatants collected on day 2, day 4 and day 5 post-transfection, respectively, under non-reducing conditions; Lane NC2 - negative control under non-reducing conditions; and lane P - multiple-tag (GenScript, Cat.No. M0101) as a positive control.

FIGs. 9A-B present images of SDS-PAGE analysis of the PD1-4-1BBL variant fusion proteins produced in Expi293F cells. Shown are PD1-4-1BBL variant fusion proteins referred to herein as “N-terminal His-tagged DSP105_var17” (SEQ ID NO: 99), “DSP105_var17” (SEQ ID NO: 97), “DSP105_var20” (SEQ ID NO: 105), “DSP105_var23” (SEQ ID NO: 113), “His-tagged DSP105_var22” (SEQ ID NO: 110), “His-tagged DSP105” (SEQ ID NO: 1), “DSP105_var24” (SEQ ID NO: 133), “DSP105_var25” (SEQ ID NO: 135) and “DSP105_var31” (SEQ ID NO: 147), under reducing (Figure 9A) or non-reducing (Figure 9B) conditions.

FIGs. 10A-C present flow cytometric analysis demonstrating DSP105_var17 (SEQ ID NO: 99) binding to PDL1 or 41BB expressing cells. FIG. 10A demonstrates expression of the indicated receptors on DLD1-PDL1 or HT1080-41BB cell lines. The surface expression level of PDL1 or 41BB was determined by immuno-staining of each cell line with the corresponding antibodies, followed by flow cytometric analysis. Figures 10B-C demonstrate binding of N-terminal His-tagged DSP105_var17 (SEQ ID NO: 99) to DLD1-PDL1 or to HT1080-41BB cell lines.

FIG. 11 presents flow cytometric analysis demonstrating expression of the 4-1BB receptor on HT1080-4-1BB cell line.

FIG. 12 presents a graph demonstrating that the N-terminal His-tagged DSP105_var17 protein (SEQ ID NO: 99), DSP105 var_17 protein (SEQ ID NO: 97), DSP105 var_21 protein (SEQ ID NO: 107) and His-tagged PD1-4-1BBL var_22 (SEQ ID NO: 110), promote 4-1BBL/4-1BB signaling as demonstrated by OD values indicating IL-8 secretion levels from HT1080-4-1BB cells, following binding of the DSP105 molecules.

FIGs. 13A-D demonstrate the predicted 3D structure of the PD1-4-1BBL variant fusion referred to herein as “DSP105_V31” (SEQ ID NO: 147). Figure 13A is a schematic 3D ribbon model. PD1 is represented in dark grey ribbons (right-hand side), 41BB-L is represented in black ribbons (left-hand side), linker segments are represented in white ribbons between the structural

elements of PD1 and 41BB-L. Figure 13B is a schematic 3D surface display model. PD1 is represented in a dark grey surface display (right-hand side), 41BB-L is represented in a black surface display (left-hand side), linker segments are represented in white ribbons between the structural elements of PD1 and 41BB-L. Figure 13C is a schematic 3D ribbon model of the fusion protein and its binding counterparts. PD1 is represented in dark grey ribbons (right-hand side), 41BB-L is represented in black ribbons (left-hand side), linker segments are represented in white ribbons between the structural elements of PD1 and 41BB-L, PD-L1 bound to PD1 is represented in grey ribbons (right-hand side) and three 41BB receptors are represented in grey ribbons in complex with 41BB-L (left-hand side). Figure 13D is a schematic 3D surface display model of the fusion protein and its binding counterparts. PD1 is represented in a dark grey surface display (right-hand side), 41BB-L is represented in a black surface display (left-hand side), linker segments are represented in white ribbons between the structural elements of PD1 and 41BB-L, PD-L1 bound to PD1 is represented in grey ribbons (right-hand side) and three 41BB receptors are represented in grey ribbons in complex with 41BB-L (left-hand side).

DESCRIPTION OF DETAILED EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to a PD1-4-1BBL variant fusion protein and methods of use thereof.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Dual Signaling Proteins (DSP), also known as Signal-Converting-Proteins (SCP), which are currently known in the art as bi-functional fusion proteins that link an extracellular portion of a type I membrane protein (extracellular amino-terminus), to an extracellular portion of a type II membrane protein (extracellular carboxyl-terminus), forming a fusion protein with two active sides.

Employing structural-functional tools, the present inventors were able to generate PD1-4-1BBL fusion proteins comprising a PD1 variant and/or a 4-1BBL variant with improved production characteristics (e.g. higher yield, lower percentage of aggregates); and which can be advantageously used for activating immune cells (via co-stimulation) in general and treating diseases that can benefit from activating immune cells (e.g. cancer) in particular.

Without being bound by theory, the following is suggested by the inventors as a mode of action of the PD1-4-1BBL fusion protein of some embodiments of the invention in the treatment of cancer as an example:

Due to the relatively high expression of PDL1 on the surface of tumor cells and in the tumor micro-environment, the PD1 moiety of the PD1-4-1BBL chimera targets the molecule to tumor and metastasis sites, leading to binding of the fusion to PDL1 within the tumor micro-environment.

Targeting the fusion protein to the tumor cells or/and tumor micro-environment facilitates an increase in PD1-4-1BBL concentration in the tumor micro-environment and subsequent oligomerization of the 4-1BBL moiety of the fusion protein at the tumor site. Since oligomerization of 4-1BBL is a necessary step for 4-1BB signaling, this 4-1BBL binding and oligomerization delivers a 4-1BB co-stimulatory signal that promotes activation of T cells, B cells, NK cells, especially Tumor-Infiltrating Lymphocytes (TILs), and other immune cells at the tumor site, to kill cancer cells.

In addition to the 4-1BBL – 4-1BB co-stimulatory signal, the binding of the fusion protein's PD1 moiety to PDL1 in the tumor site competes with the endogenous PD1 expressed on T cells; thus, removing the inhibition on T cells and further contributing to the activation of TILs in the tumor micro-environment.

The above activities of PD1-4-1BBL are expected to lead to a synergistic effect on the activation of TILs within the tumor micro-environment, which is expected to be a more specific and more robust effect as compared to the effect of each moiety separately, as well as when using the two different moieties thereof in combination.

Thus, according to an aspect of the present invention, there is provided a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL amino acid sequence, wherein said PD1 amino acid sequence:

(a) is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or is 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and does not comprise an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and/or

(b) comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and/or
wherein said 4-1BBL amino acid sequence:

18

(aa) is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and does not comprise an amino acid segment G198-E205
 5 corresponding to SEQ ID NO: 3, is 170 – 182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and does not comprise an amino acid segment A1-E23 corresponding to SEQ ID NO: 3 or is 184 amino acids in length having at least 80 % identity to SEQ ID NO: 123; and/or

(bb) comprises three repeats of a 4-1BBL amino acid sequence;

and wherein said fusion protein is capable of at least one of:

- 10 (i) binding PDL1 and 4-1BB;
 (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
 (iii) co-stimulating immune cells expressing said 4-1BB.

According to an alternative or an additional aspect of the present invention, there is provided a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL
 15 amino acid sequence, wherein said PD1 amino acid sequence:

(a) is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2 and/or

- 20 (b) comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and/or

wherein said 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25; and wherein said fusion protein is capable of at least one of:

- 25 (i) binding PDL1 and 4-1BB;
 (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
 (iii) co-stimulating immune cells expressing said 4-1BB.

According to an alternative or an additional aspect of the present invention, there is provided a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL
 30 amino acid sequence, wherein said PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2 and/or wherein said 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the

group consisting of SEQ ID NOs: 22-25; and wherein said fusion protein is capable of at least one of:

- (i) binding PDL1 and 4-1BB;
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
- (iii) co-stimulating immune cells expressing said 4-1BB.

According to an alternative or an additional aspect of the present invention, there is provided a PD1-4-1BBL fusion protein comprising a (GGGGS)_n(n=1-4) linker between said PD1 and said 4-1BBL, wherein said fusion protein is capable of at least one of:

- (i) binding PDL1 and 4-1BB;
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
- (iii) co-stimulating immune cells expressing said 4-1BB.

According to an alternative or an additional aspect of the present invention, there is provided an isolated polypeptide comprising a PD1 amino acid sequence, wherein said PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or is 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and does not comprise an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and optionally comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and wherein said polypeptide is capable of binding PDL1.

According to an alternative or an additional aspect of the present invention, there is provided an isolated polypeptide comprising a PD1 amino acid sequence, wherein said PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and optionally comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and wherein said polypeptide is capable of binding PDL1.

According to an alternative or an additional aspect of the present invention, there is provided an isolated polypeptide comprising a PD1 amino acid sequence, wherein said PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and wherein said polypeptide is capable of binding PDL1.

According to an alternative or an additional aspect of the present invention, there is provided an isolated polypeptide comprising a 4-1BBL amino acid sequence, wherein said 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and does not comprise an amino acid segment G198-E205 corresponding to SEQ ID NO: 3, or is 170 – 182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and does not comprise an amino acid segment A1-E23 corresponding to SEQ ID NO: 3; and optionally comprises three repeats of said 4-1BBL amino acid sequence; and wherein said polypeptide is capable of at least one of:

- (i) binding 4-1BB,
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
- (iii) co-stimulating immune cells expressing said 4-1BB.

According to an alternative or an additional aspect of the present invention, there is provided an isolated polypeptide comprising a 4-1BBL amino acid sequence, wherein said 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25; and wherein said polypeptide is capable of at least one of:

- (i) binding 4-1BB,
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
- (iii) co-stimulating immune cells expressing said 4-1BB.

As used herein the term “PD1 (Programmed Death 1, also known as CD279)” refers to the polypeptide of the *PDCD1* gene (Gene ID 5133) or a functional homolog e.g., functional fragment thereof. According to specific embodiments, the term “PD1” refers to a functional homolog of PD1 polypeptide. According to specific embodiments, PD1 is human PD1. According to a specific embodiment, the PD1 protein refers to the human protein, such as provided in the following GenBank Number NP_005009.

Two ligands for PD-1 have been identified so far, PDL1 and PDL2 (also known as B7-DC). According to a specific embodiment, the PDL1 protein refers to the human protein, such as provided in the following GenBank Number NP_001254635 and NP_054862. According to a specific embodiment, the PDL2 protein refers to the human protein, such as provided in the following GenBank Number NP_079515.

As use herein, the phrase “functional homolog” or “functional fragment” when related to PD1 refers to a portion of the polypeptide which maintains the activity of the full length PD1 e.g., PD-L1 binding.

Assays for testing binding are well known in the art and include, but not limited to flow cytometry, 5 BiaCore, bio-layer interferometry Blitz® assay, HPLC.

According to specific embodiments, the PD1 binds PD-L1 with a K_d of 1 nM – 100 μ M, 10-nM – 10 μ M, 100 nM – 100 μ M, 200 nM – 10 μ M, as determined by SPR analysis, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 binds PDL1 with a K_d of about 270 nM as 10 determined by SPR analysis.

According to specific embodiments, the PD1 binds PDL1 with a K_d of about 8-9 μ M as determined by SPR analysis,

According to specific embodiments, the PD1 comprises an extracellular domain of said PD1 or a functional fragment thereof.

15 According to specific embodiments, PD1 amino acid sequence comprises SEQ ID NO: 27.

According to specific embodiments, PD1 amino acid sequence consists of SEQ ID NO: 27.

According to specific embodiments, PD1 nucleic acid sequence comprises SEQ ID NO: 28.

According to specific embodiments, PD1 nucleic acid sequence consists of SEQ ID NO: 28.

According to specific embodiments, PD1 amino acid sequence comprises SEQ ID NO: 2.

20 According to specific embodiments, PD1 amino acid sequence consists of SEQ ID NO: 2.

According to specific embodiments, PD1 nucleic acid sequence comprises SEQ ID NO: 29.

According to specific embodiments, PD1 nucleic acid sequence consists of SEQ ID NO: 29.

According to specific embodiments, PD1 amino acid sequence comprises SEQ ID NO: 30 or SEQ ID NO: 31.

25 According to specific embodiments, PD1 amino acid sequence consists of SEQ ID NO: 30 or SEQ ID NO: 31.

The term “PD1” also encompasses functional homologues (naturally occurring or synthetically/recombinantly produced), which exhibit the desired activity (*i.e.*, binding PD-L1 and/or PD-L2). Such homologues can be, for example, at least 70 %, at least 75 %, at least 80 %, 30 at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to the polypeptide SEQ ID NO: 2, 27, 30 or 31; or at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least

87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the polynucleotide sequence encoding same (as further described hereinbelow).

According to specific embodiments the PD1 functional homologues are at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to the polypeptide SEQ ID NO: 2, 27, 30 or 31; or at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the polynucleotide sequence encoding same (as further described hereinbelow).

As used herein, "identity" or "sequence identity" refers to global identity, *i.e.*, an identity over the entire amino acid or nucleic acid sequences disclosed herein and not over portions thereof.

Sequence identity or homology can be determined using any protein or nucleic acid sequence alignment algorithm such as Blast, ClustalW, and MUSCLE.

The homolog may also refer to an ortholog, a deletion, insertion, or substitution variant, including an amino acid substitution, as further described hereinbelow.

According to specific embodiments, the PD1 polypeptide may comprise conservative and non-conservative amino acid substitutions (also referred to herein as mutations). Such substitution are known in the art and disclosed e.g. in Maute et al. PNAS, 2015 Nov 24;112(47):E6506-14; Ju Yeon et al. Nature Communications 2016 volume 7, Article number: 13354 (DOI: 10.1038/ncomms13354); and Zack KM et al. Structure. 2015 23(12): 2341-2348 (DOI:10.1016/j.str.2015.09.010), the contents of which are fully incorporated herein by reference.

When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g. charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences which differ by such conservative substitutions are considered to have "sequence similarity" or "similarity". Means for making this adjustment are well-known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., according to the algorithm of Henikoff S and Henikoff JG. [Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. U.S.A. 1992, 89(22): 10915-9].

23

According to specific embodiments, one or more amino acid mutations are located at an amino acid residue selected from: V39, L40, N41, Y43, R44, M45, S48, N49, Q50, T51, D52, K53, A56, Q63, G65, Q66, V72, H82, M83, R90, Y96, L97, A100, S102, L103, A104, P105, K106, and A107 corresponding to the PD1 amino acid sequence set forth in SEQ ID NO: 31. According to specific

5 embodiments, one or more amino acid mutations are located at an amino acid residue selected from: V39, L40, N41, Y43, R44, M45, S48, N49, Q50, T51, D52, K53, A56, Q63, G65, Q66, C68, V72, H82, M83, R90, Y96, L97, A100, S102, L103, A104, P105, K106, and A107 corresponding to the PD1 amino acid sequence set forth in SEQ ID NO: 31.

According to specific embodiments, one or more amino acid changes are selected from the

10 group consisting of: (1) V39H or V39R; (2) L40V or L40I; (3) N41I or N41V; (4) Y43F or Y43H; (5) R44Y or R44L; (6) M45Q, M45E, M45L, or M45D; (7) S48D, S48L, S48N, S48G, or S48V; (8) N49C, N49G, N49Y, or N49S; (9) Q50K, Q50E, or Q50H; (10) T51V, T51L, or T51A; (11) D52F, D52R, D52Y, or D52V; (12) K53T or K53L; (13) A56S or A56L; (14) Q63T, Q63I, Q63E, Q63L, or Q63P; (15) G65N, G65R, G65I, G65L, G65F, or G65V; (16) Q66P; (17) V72I; (18)

15 H82Q; (19) M83L or M83F; (20) R90K; (21) Y96F; (22) L97Y, L97V, or L97I; (23) A100I or A100V; (24) S102T or S102A; (25) L103I, L103Y, or L103F; (26) A104S, A104H, or A104D; (27) P105A; (28) K106G, K106E, K106I, K106V, K106R, or K106T; and (29) A107P, A107I, or A107V corresponding to the PD1 amino acid sequence set forth in SEQ ID NO: 31.

According to specific embodiments, one or more amino acid changes are selected from the

20 group consisting of: (1) V39H or V39R; (2) L40V or L40I; (3) N41I or N41V; (4) Y43F or Y43H; (5) R44Y or R44L; (6) M45Q, M45E, M45L, or M45D; (7) S48D, S48L, S48N, S48G, or S48V; (8) N49C, N49G, N49Y, or N49S; (9) Q50K, Q50E, or Q50H; (10) T51V, T51L, or T51A; (11) D52F, D52R, D52Y, or D52V; (12) K53T or K53L; (13) A56S or A56L; (14) Q63T, Q63I, Q63E, Q63L, or Q63P; (15) G65N, G65R, G65I, G65L, G65F, or G65V; (16) Q66P; (17) C68S (18),

25 V72I; (19) H82Q; (20) M83L or M83F; (21) R90K; (22) Y96F; (23) L97Y, L97V, or L97I; (24) A100I or A100V; (25) S102T or S102A; (26) L103I, L103Y, or L103F; (27) A104S, A104H, or A104D; (28) P105A; (29) K106G, K106E, K106I, K106V, K106R, or K106T; and (30) A107P, A107I, or A107V corresponding to the PD1 amino acid sequence set forth in SEQ ID NO: 31.

According to specific embodiments, an amino acid mutation is located at an amino acid residue

30 C73 corresponding to the PD1 amino acid sequence set forth in SEQ ID NO: 2 (e.g. equivalent to an amino acid residue C68 corresponding to the PD1 amino acid sequence set forth in SEQ ID NO: 31).

According to specific embodiments, the PD1 polypeptide may comprise a C to S amino acid modification in a position corresponding to amino acid residue 73 of the PD1 amino acid sequence

set forth in SEQ ID NO: 2 (e.g. equivalent to amino acid residue 68 of the PD1 amino acid sequence set forth in SEQ ID NO: 31).

Thus, according to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 73.

5 According to specific embodiments, PD1 amino acid sequence consists of SEQ ID NO: 73.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO: 74.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 74.

10 As used herein, the phrase “corresponding to PD1 amino acid sequence as set forth in SEQ ID NO: 31”, “corresponding to SEQ ID NO: 31”, “corresponding to PD1 amino acid sequence as set forth in SEQ ID NO: 2” or “corresponding to SEQ ID NO: 2”, intends to include the corresponding amino acid residue relative to any other PD1 amino acid sequence.

Additional description on conservative amino acid and non-conservative amino acid
15 substitutions is further provided hereinbelow.

The PD1 of some embodiments of the present invention is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to the polypeptide
20 SEQ ID NO: 18-21; or at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the polynucleotide sequence encoding same (as further described hereinbelow), each possibility represents a separate embodiment of the present invention.

25 According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 and 115.

According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119.

30 According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 117 and 119.

According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93.

According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21.

According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to SEQ ID NO: 18.

5 According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to SEQ ID NO: 19.

According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to SEQ ID NO: 20.

10 According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to SEQ ID NO: 21.

According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to SEQ ID NO: 85.

According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to SEQ ID NO: 89.

15 According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to SEQ ID NO: 93.

According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to SEQ ID NO: 115.

20 According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to SEQ ID NO: 117.

According to specific embodiments, the PD1 amino acid sequence has at least 80 % identity to SEQ ID NO: 119.

25 According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 and 115.

According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119.

According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 117 and 119.

30 According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93.

According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21.

According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to SEQ ID NO: 18.

According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to SEQ ID NO: 19.

5 According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to SEQ ID NO: 20.

According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to SEQ ID NO: 21.

10 According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to SEQ ID NO: 85.

According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to SEQ ID NO: 89.

According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to SEQ ID NO: 93.

15 According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to SEQ ID NO: 115.

According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to SEQ ID NO: 117.

20 According to specific embodiments, the PD1 amino acid sequence has at least 85 % identity to SEQ ID NO: 119.

According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 and 115.

25 According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119.

According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 117 and 119.

30 According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93.

According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21.

According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to SEQ ID NO: 18.

According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to SEQ ID NO: 19.

According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to SEQ ID NO: 20.

5 According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to SEQ ID NO: 21.

According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to SEQ ID NO: 85.

10 According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to SEQ ID NO: 89.

According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to SEQ ID NO: 93.

According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to SEQ ID NO: 115.

15 According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to SEQ ID NO: 117.

According to specific embodiments, the PD1 amino acid sequence has at least 90 % identity to SEQ ID NO: 119.

20 According to specific embodiments, the PD1 amino acid sequence has at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 and 115.

25 According to specific embodiments, the PD1 amino acid sequence has at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119.

30 According to specific embodiments, the PD1 amino acid sequence has at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 117 and 119.

According to specific embodiments, the PD1 amino acid sequence has at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID

NO: 18-21, 85, 89 and 93, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence has at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence has at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21.

According to specific embodiments, the PD1 amino acid sequence has at least 95 % identity to SEQ ID NO: 18.

According to specific embodiments, the PD1 amino acid sequence has at least 95 % identity to SEQ ID NO: 19.

According to specific embodiments, the PD1 amino acid sequence has at least 95 % identity to SEQ ID NO: 20.

According to specific embodiments, the PD1 amino acid sequence has at least 95 % identity to SEQ ID NO: 21.

According to specific embodiments, the PD1 amino acid sequence has at least 95 % identity to SEQ ID NO: 85.

According to specific embodiments, the PD1 amino acid sequence has at least 95 % identity to SEQ ID NO: 89.

According to specific embodiments, the PD1 amino acid sequence has at least 95 % identity to SEQ ID NO: 93.

According to specific embodiments, the PD1 amino acid sequence has at least 95 % identity to SEQ ID NO: 115.

According to specific embodiments, the PD1 amino acid sequence has at least 95 % identity to SEQ ID NO: 117.

According to specific embodiments, the PD1 amino acid sequence has at least 95 % identity to SEQ ID NO: 119.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the

group consisting of SEQ ID NO: 18, 117 and 119, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 117 and 119, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 18.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 19.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 20.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 21.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 85.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 89.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 93.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 115.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 117.

According to specific embodiments, the PD1 amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 119.

According to specific embodiments, the PD1 amino acid sequence does not comprise amino acid segment P1 – L5 corresponding to SEQ ID NO: 2.

According to specific embodiments, the PD1 amino acid sequence does not comprise amino acid segment F146 – V150 corresponding to SEQ ID NO: 2.

5 According to specific embodiments, the PD1 amino acid sequence does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2.

According to specific embodiments, the PD1 amino acid sequence does not comprise any of amino acid residues P1 – L5 corresponding to SEQ ID NO: 2.

10 According to specific embodiments, the PD1 amino acid sequence does not comprise any of amino acid residues F146 – V150 corresponding to SEQ ID NO: 2.

According to specific embodiments, the PD1 amino acid sequence does not comprise any of amino acid residues P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2.

According to specific embodiments, the PD1 amino acid sequence does not comprise SEQ ID NO: 8 or any fragment thereof.

15 According to specific embodiments, the PD1 amino acid sequence does not comprise SEQ ID NO: 8.

According to specific embodiments, the PD1 amino acid sequence does not comprise SEQ ID NO: 9 or any fragment thereof.

20 According to specific embodiments, the PD1 amino acid sequence does not comprise SEQ ID NO: 9.

According to specific embodiments, the PD1 amino acid sequence does not comprise SEQ ID NO: 8 and 9 or any fragment thereof.

According to specific embodiments, the PD1 amino acid sequence does not comprise SEQ ID NO: 8 and 9.

25 According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 or 115.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 18, 117 or 119.

30 According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 117 or 119.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 18, 19, 20, 21, 85, 89 or 93.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 75, 83, 87, 91 or 95.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 18, 19, 20 or 21.

5 According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 18 or 21.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 18.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 19.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 20.

10 According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 21.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 85.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 89.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 93.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 75.

15 According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 79.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 81.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 83.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 87.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 91.

20 According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 95.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 115.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 117.

25 According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 119.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 or 115.

30 According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 18, 117 or 119.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 117 or 119.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 18, 19, 20, 21, 85, 89 or 93.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 75, 83, 87, 91 or 95.

5 According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 18, 19, 20 or 21.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 18 or 21.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 18.

10 According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 19.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 20.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 21.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 85.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 89.

15 According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 93.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 75.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 79.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 81.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 83.

20 According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 87.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 91.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 95.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 115.

25 According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 117.

According to specific embodiments, the PD1 amino acid sequence consists of SEQ ID NO: 119.

30 According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 118 and/or 120, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94 and/or 96, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 76, 80, 82, 84, 86, 88, 90, 92, 94 and/or 96, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 76, 80, 82, 84, 88, 92 and/or 96, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 32, 33, 34 and/or 35, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 32, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100

% identity to SEQ ID NO: 33, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 34, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 35, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 76, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 80, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 82, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least

86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 84, each possibility represents a separate embodiment of the present invention.

5 According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 86, each possibility represents a separate embodiment of the present
10 invention.

 According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100
15 % identity to SEQ ID NO: 88, each possibility represents a separate embodiment of the present invention.

 According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least
20 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 90, each possibility represents a separate embodiment of the present invention.

 According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least
25 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 92, each possibility represents a separate embodiment of the present invention.

 According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least
30 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 94, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 96, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 116, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 118, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 120, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 118 and/or 120.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94 and/or 96.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 32, 33, 34 and/or 35.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 32.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 33.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 34.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 35.

5 According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 76.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 80.

10 According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 82.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 84.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 86.

15 According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 88.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 90.

20 According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 92.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 94.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 96.

25 According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 116.

According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 118.

30 According to specific embodiments, the PD1 nucleic acid sequence has at least 80 % identity to SEQ ID NO: 120.

According to specific embodiments, the PD1 nucleic acid sequence has at least 85 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 118 and/or 120.

According to specific embodiments, the PD1 nucleic acid sequence has at least 85 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94 and/or 96.

According to specific embodiments, the PD1 nucleic acid sequence has at least 85 % identity to SEQ ID NO: 32, 33, 34 and/or 35.

According to specific embodiments, the PD1 nucleic acid sequence has at least 90 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 118 and/or 120.

5 According to specific embodiments, the PD1 nucleic acid sequence has at least 90 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94 and/or 96.

According to specific embodiments, the PD1 nucleic acid sequence has at least 90 % identity to SEQ ID NO: 32, 33, 34 and/or 35.

10 According to specific embodiments, the PD1 nucleic acid sequence has at least 95 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 118 and/or 120.

According to specific embodiments, the PD1 nucleic acid sequence has at least 95 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94 and/or 96.

According to specific embodiments, the PD1 nucleic acid sequence has at least 95 % identity to SEQ ID NO: 32, 33, 34 and/or 35.

15 According to specific embodiments, the PD1 nucleic acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 118 and/or 120, each possibility represents a separate embodiment of the present invention.

20 According to specific embodiments, the PD1 nucleic acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94 and/or 96, each possibility represents a separate embodiment of the present invention.

25 According to specific embodiments, the PD1 nucleic acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 32, 33, 34 and/or 35, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 118 or 120.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94 or 96.

30 According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO: 32, 33, 34 or 35.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO: 32.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
33.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
34.

5 According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
35.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
76.

10 According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
80.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
82.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
84.

15 According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
86.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
88.

20 According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
90.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
92.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
94.

25 According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
96.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
116.

30 According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
118.

According to specific embodiments, the PD1 nucleic acid sequence comprises SEQ ID NO:
120.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO:
32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 180 or 120.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 32, 33, 34, 35, 76, 80, 82, 84, 86, 88, 90, 92, 94 or 96.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 32, 33, 34 and/or 35.

5 According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 32.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 33.

10 According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 34.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 35.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 76.

15 According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 80.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 82.

20 According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 84.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 86.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 88.

25 According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 90.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 92.

30 According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 94.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 96.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 116.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 118.

According to specific embodiments, the PD1 nucleic acid sequence consists of SEQ ID NO: 120.

5 According to specific embodiments, PD1 amino acid sequence comprises 100-200 amino acids, 120-180 amino acids, 120-160, 130-170 amino acids, 130-160, 130-150, 140-160 amino acids, 145-155 amino acids, each possibility represents a separate embodiment of the present invention.

10 According to specific embodiments, PD1 amino acid sequence is 123-166 amino acids in length.

According to specific embodiment, PD1 amino acid sequence is 156 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 145-155 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 150 amino acids in length.

15 According to specific embodiment, PD1 amino acid sequence comprises less than 150 amino acids in length.

According to specific embodiments, PD1 amino acid sequence comprises less than 149 amino acids in length.

20 According to specific embodiments, PD1 amino acid sequence comprises less than 148, less than 147, less than 146, less than 145, less than 144, less than 143, less than 142, less than 141 amino acids in length, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, PD1 amino acid sequence is 138-145 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 145 amino acids in length.

25 According to specific embodiments, PD1 amino acid sequence is 143 amino acids in length.

According to specific embodiments, the PD1 amino acid sequence is no more than 140 amino acids in length.

30 According to specific embodiments, PD1 amino acid sequence is at least 123, at least 124, at least 125, at least 126, at least 127, at least 128, at least 129, at least 130, at least 133, at least 137, at least 138 amino acids in length, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, PD1 amino acid sequence is at least 123, at least 124, at least 125, at least 126, at least 127, at least 128, at least 129, at least 130 amino acids in length, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, PD1 amino acid sequence comprises 123 – 148 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is at least 126 amino acids in length.

5 According to specific embodiments, PD1 amino acid sequence is 126 – 148 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 123 – 140 amino acids in length.

10 According to specific embodiments, PD1 amino acid sequence is 126 – 140 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 127 – 140 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 128 – 140 amino acids in length.

15 According to specific embodiments, PD1 amino acid sequence is 130 – 140 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 130 – 135 amino acids in length.

20 According to specific embodiments, PD1 amino acid sequence comprises 135 – 140 amino acids in length.

According to specific embodiments, PD1 amino acid sequence comprises 126 – 130 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 127 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 128 amino acids in length.

25 According to specific embodiments, PD1 amino acid sequence is 133 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 135 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 136 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 137 amino acids in length.

According to specific embodiments, PD1 amino acid sequence is 138 amino acids in length.

30 According to specific embodiments, PD1 amino acid sequence is 140 amino acids in length.

As used herein the term “4-1BBL (also known as CD137L and TNFSF9)” refers to the polypeptide of the TNFSF9 gene (Gene ID 8744) or a functional homolog e.g., functional fragment thereof. According to specific embodiments, the term “4-1BBL” refers to a functional homolog of 4-1BBL polypeptide. According to specific embodiments, 4-1BBL is human 4-1BBL.

According to a specific embodiment, the 4-1BBL protein refers to the human protein, such as provided in the following GenBank Number NP_003802.

As use herein, the phrase “functional homolog” or “functional fragment” when related to 4-1BBL, refers to a portion of the polypeptide which maintains the activity of the full length 4-1BBL e.g., (i) binding 4-1BB, (ii) activating 4-1BB signaling pathway, (iii) activating immune cells expressing 4-1BB, (iv) forming a homotrimer.

According to specific embodiments, the functional homolog when related to 4-1BBL is capable of (i), (ii), (iii), (i)+(ii), (i)+(iii), (ii)+(iii).

According to specific embodiments, the functional homolog when related to 4-1BBL is capable of (i)+(ii)+(iii).

According to specific embodiments, the functional homolog when related to 4-1BBL is capable of (iv), (i)+(iv), (ii)+(iv), (iii)+(iv), (i)+(ii)+(iv), (i)+(iii)+(iv), (ii)+(iii)+(iv).

According to specific embodiments, the functional homolog when related to 4-1BBL is capable of (i)+(ii)+(iii)+(iv).

According to a specific embodiment, the 4-1BB protein refers to the human protein, such as provided in the following GenBank Number NP_001552.

Assays for testing binding are well known in the art and are further described hereinabove and below.

According to specific embodiments, the 4-1BBL binds 4-1BB with a Kd of about 0.1 – 1000 nM, 0.1 – 100 nM, 1-100 nM, or 55.2 nM as determined by SPR, each possibility represents a separate embodiment of the claimed invention.

Assays for testing trimerization are well known in the art and include, but not limited to NATIVE-PAGE, SEC-HPLC 2D gels, gel filtration, SEC-MALS, Analytical ultracentrifugation (AUC) Mass spectrometry (MS), capillary gel electrophoresis (CGE).

As used herein the terms “activating” or “activation” refer to the process of stimulating an immune cell (e.g. T cell, B cell, NK cell, phagocytic cell) that results in cellular proliferation, maturation, cytokine production, phagocytosis and/or induction of regulatory or effector functions.

According to specific embodiments, activating comprises co-stimulating.

As used herein the term “co-stimulating” or “co-stimulation” refers to transmitting a secondary antigen independent stimulatory signal (e.g. 4-1BB signal) resulting in activation of the immune cell.

According to specific embodiments, activating comprises suppressing an inhibitory signal (e.g. PDL1 signal) resulting in activation of the immune cell.

Methods of determining signaling of a stimulatory or inhibitory signal are well known in the art and also disclosed in the Examples section which follows, and include, but are not limited to, binding assay using e.g. BiaCore, HPLC or flow cytometry, enzymatic activity assays such as kinase activity assays, and expression of molecules involved in the signaling cascade using e.g. PCR, Western blot, immunoprecipitation and immunohistochemistry. Additionally or alternatively, determining transmission of a signal (co-stimulatory or inhibitory) can be effected by evaluating immune cell activation or function. Methods of evaluating immune cell activation or function are well known in the art and include, but are not limited to, proliferation assays such as CFSE staining, MTS, Alamar blue, BRDU and thymidine incorporation, cytotoxicity assays such as CFSE staining, chromium release, Calcin AM, cytokine secretion assays such as intracellular cytokine staining, ELISPOT and ELISA, expression of activation markers such as CD25, CD69, CD137, CD107a, PD1, and CD62L using flow cytometry.

According to specific embodiments, determining the signaling activity or activation is effected in-vitro or ex-vivo e.g. in a mixed lymphocyte reaction (MLR), as further described hereinbelow.

For the same culture conditions the signaling activity or the immune cell activation or function are generally expressed in comparison to the signaling, activation or function in a cell of the same species but not contacted with the PF1-4-1BBL fusion protein, a polynucleotide encoding same or a host cell encoding same; or contacted with a vehicle control, also referred to as control.

According to specific embodiments, the 4-1BBL comprises an extracellular domain of said 4-1BBL or a functional fragment thereof.

According to specific embodiments, 4-1BBL amino acid sequence comprises SEQ ID NO: 36.

According to specific embodiments, 4-1BBL amino acid sequence consists of SEQ ID NO: 36.

According to specific embodiments, 4-1BBL nucleic acid sequence comprises SEQ ID NO: 37.

According to specific embodiments, 4-1BBL nucleic acid sequence consists of SEQ ID NO: 37.

According to specific embodiments, 4-1BBL amino acid sequence comprises SEQ ID NO: 3.

According to specific embodiments, 4-1BBL amino acid sequence consists of SEQ ID NO: 3.

According to specific embodiments, 4-1BBL nucleic acid sequence comprises SEQ ID NO: 38.

According to specific embodiments, 4-1BBL nucleic acid sequence consists of SEQ ID NO: 38.

5 The term “4-1BBL” also encompasses functional homologues (naturally occurring or synthetically/recombinantly produced), which exhibit the desired activity (as defined hereinabove). Such homologues can be, for example, at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, 10 at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to the polypeptide SEQ ID NO: 3, 36; or at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the 15 polynucleotide sequence encoding same (as further described hereinbelow).

According to specific embodiments the 4-1BBL functional homologues are at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to the polypeptide SEQ ID NO: 3, 36; or at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the polynucleotide sequence encoding same (as further described 20 hereinbelow).

According to specific embodiments, the 4-1BBL polypeptide may comprise conservative amino acid substitutions, as further described hereinabove and below.

The 4-1BBL of some embodiments of the present invention is at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical or homologous to the polypeptide 25 SEQ ID NO: 22, 23, 24 and/or 25; or at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identical to the polynucleotide sequence encoding same (as further described hereinbelow), each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, each 30 possibility represents a separate embodiment of the present invention.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22, 23, 24 and/or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22, 23 and/or 24.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 23.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 24.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 25.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise the amino acid segment A1 – V6 corresponding to SEQ ID NO: 3.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise the amino acid segment A1 – G14 corresponding to SEQ ID NO: 3.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise any of amino acid residues A1 – V6 or A1 – G14 corresponding to SEQ ID NO: 3.

As used herein, the phrase “corresponding to SEQ ID NO: 3” intends to include the corresponding amino acid residue relative to any other 4-1BBL amino acid sequence.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise SEQ ID NO: 6 or 7 or any fragment thereof.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise SEQ ID NO: 6 or 7.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 22, 23, 24 or 25.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 22, 23 or 24.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 22 or 23.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 22.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 23.

According to specific embodiments, the 4-1BBL α amino acid sequence comprises SEQ ID NO: 24.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 25.

5 According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NO: 22, 23, 24 or 25.

According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NO: 22, 23 or 24.

10 According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NO: 22 or 23.

According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NO: 22.

According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NO: 23.

15 According to specific embodiments, the 4-1BBL α amino acid sequence consists of SEQ ID NO: 24.

According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NO: 25.

20 According to specific embodiments, the 4-1BBL amino acid sequence has at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 127 and 129, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 85 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 127 and 129, each possibility represents a separate embodiment of the present invention.

25 According to specific embodiments, the 4-1BBL amino acid sequence has at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 127 and 129, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 127 and 129, each possibility represents a separate embodiment of the present invention.

30 According to specific embodiments, the 4-1BBL amino acid sequence has at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 127 and 129, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 127 and 129, each possibility represents a separate embodiment of the present invention.

5 According to specific embodiments, the 4-1BBL amino acid sequence does not comprise the amino acid segment G198 – E205 corresponding to SEQ ID NO: 3.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise any of amino acid residues G198 – E205 corresponding to SEQ ID NO: 3.

10 According to specific embodiments, the 4-1BBL amino acid sequence does not comprise SEQ ID NO: 166 or any fragment thereof.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise SEQ ID NO: 166.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NOs: 127 or 129.

15 According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NOs: 127 or 129.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 80 % identity to SEQ ID NO: 125.

20 According to specific embodiments, the 4-1BBL amino acid sequence has at least 85 % identity SEQ ID NO: 125.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 90 % identity to SEQ ID NO: 125.

25 According to specific embodiments, the 4-1BBL amino acid sequence has at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 125.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 95 % identity to SEQ ID NO: 125.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 125.

30 According to specific embodiments, the 4-1BBL amino acid sequence does not comprise the amino acid segment A1-E23 corresponding to SEQ ID NO: 3.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise any of amino acid residues A1-E23 corresponding to SEQ ID NO: 3.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise SEQ ID NO: 167 or any fragment thereof.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise SEQ ID NO: 167.

5 According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 125.

According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NO: 125.

10 According to specific embodiments, the 4-1BBL amino acid sequence has at least 80 % identity to SEQ ID NO: 123.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 85 % identity SEQ ID NO: 123.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 90 % identity to SEQ ID NO: 123.

15 According to specific embodiments, the 4-1BBL amino acid sequence has at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 123.

According to specific embodiments, the 4-1BBL amino acid sequence has at least 95 % identity to SEQ ID NO: 123.

20 According to specific embodiments, the 4-1BBL amino acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 123.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 123.

25 According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NO: 123.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 39, 40, 41 and/or 42, each possibility represents a separate embodiment of the present invention.

30

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 95 % identity to SEQ ID NO: 39, 40, 41 and/or 42.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 39, 40, 41 and/or 42.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 39 and/or 40.

According to specific embodiments, the 4-1BBL nucleic acid sequence comprises SEQ ID NO: 39, 40, 41 or 42.

According to specific embodiments, the 4-1BBL nucleic acid sequence comprises SEQ ID NO: 39, 40 or 41.

According to specific embodiments, the 4-1BBL nucleic acid sequence comprises SEQ ID NO: 39 or 40.

According to specific embodiments, the 4-1BBL nucleic acid sequence comprises SEQ ID NO: 39.

According to specific embodiments, the 4-1BBL nucleic acid sequence comprises SEQ ID NO: 40.

According to specific embodiments, the 4-1BBL α nucleic acid sequence comprises SEQ ID NO: 41.

According to specific embodiments, the 4-1BBL nucleic acid sequence comprises SEQ ID NO: 42.

According to specific embodiments, the 4-1BBL nucleic acid sequence consists of SEQ ID NO: 39, 40, 41 or 42.

According to specific embodiments, the 4-1BBL nucleic acid sequence consists of SEQ ID NO: 39, 40 or 41.

According to specific embodiments, the 4-1BBL nucleic acid sequence consists of SEQ ID NO: 39 or 40.

According to specific embodiments, the 4-1BBL nucleic acid sequence consists of SEQ ID NO: 39.

According to specific embodiments, the 4-1BBL nucleic acid sequence consists of SEQ ID NO: 40.

According to specific embodiments, the 4-1BBL nucleic acid sequence consists of SEQ ID NO: 41.

According to specific embodiments, the 4-1BBL nucleic acid sequence consists of SEQ ID NO: 42.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 128 and/or 130, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 95 % identity to SEQ ID NO: 128 and/or 130.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 128 and/or 130.

According to specific embodiments, the 4-1BBL nucleic acid sequence comprises SEQ ID NO: 128 or 130.

According to specific embodiments, the 4-1BBL nucleic acid sequence consists of SEQ ID NO: 128 or 130.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 126.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 95 % identity to SEQ ID NO: 126.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 126.

According to specific embodiments, the 4-1BBL nucleic acid sequence comprises SEQ ID NO: 126.

According to specific embodiments, the 4-1BBL nucleic acid sequence consists of SEQ ID NO: 126.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 124.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 95 % identity to SEQ ID NO: 124.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 124.

According to specific embodiments, the 4-1BBL nucleic acid sequence comprises SEQ ID NO: 124.

5 According to specific embodiments, the 4-1BBL nucleic acid sequence consists of SEQ ID NO: 124.

According to specific embodiments, 4-1BBL amino acid sequence comprises 100-300 amino acids, 150-250 amino acids, 100-250 amino acids, 150-220 amino acids, 180-220 amino acids, 180 – 210 amino acids, 185 – 205 amino acids, 190-210 amino acids, each possibility represents
10 a separate embodiment of the present invention.

According to specific embodiments, 4-1BBL amino acid sequence is 190-210 amino acids in length.

According to specific embodiments, 4-1BBL amino acid sequence is 204 amino acids in length.

15 According to specific embodiments, 4-1BBL amino acid sequence is 185 – 202 amino acids in length.

According to specific embodiments, 4-1BBL amino acid sequence is 185 – 200 amino acids in length.

20 According to specific embodiments, 4-1BBL amino acid sequence is 185 – 199 amino acids in length.

According to specific embodiments, 4-1BBL amino acid sequence is 170 – 197 amino acids in length.

According to specific embodiments, 4-1BBL amino acid sequence is 170 – 182 amino acids in length.

25 According to specific embodiments, 4-1BBL amino acid sequence is 184 amino acids in length.

According to specific embodiments, 4-1BBL amino acid sequence is 185, 191, 197 or 199 amino acids in length, each possibility represents a separate embodiment of the present invention.

30 According to specific embodiments, 4-1BBL amino acid sequence is 184 amino acids in length.

According to specific embodiments, 4-1BBL amino acid sequence is 183 amino acids in length.

According to specific embodiments, 4-1BBL amino acid sequence is 182 amino acids in length.

According to specific embodiments, 4-1BBL amino acid sequence is 176 amino acids in length.

According to specific embodiments, the 4-1BBL amino acid sequence comprised in the PD1-4-1BBL fusion protein or the 4-1BBL polypeptide disclosed herein comprises three repeats of a 4-1BBL amino acid sequence.

According to specific embodiments, each of the three repeats is capable of at least one of: (i) binding 4-1BB, (ii) activating 4-1BB signaling pathway, (iii) activating immune cells expressing 4-1BB, (iv) forming a homotrimer.

According to specific embodiments, the 4-1BBL amino acid sequence does not comprise a linker between each of said three repeats of said 4-1BBL amino acid sequence.

According to other specific embodiments, the 4-1BBL amino acid sequence comprises a linker between each of said three repeats of said 4-1BBL amino acid sequence. Any linker known in the art can be used with specific embodiments of the invention. Non-limiting examples of linkers that can be used are described in details hereinbelow.

According to a specific embodiment, the linker is a (GGGGS)₂+GGGG (SEQ ID NO: 121) linker.

According to specific embodiments, the repeated sequence can be any of the 4-1BBL as defined herein.

According to specific embodiments, the three repeats have an identical 4-1BBL amino acid sequence.

According to other specific embodiments, the three repeats are distinct, i.e. have different 4-1BBL amino acid sequences.

According to other specific embodiments, two of the three repeats have an identical 4-1BBL amino acid sequence.

According to specific embodiments, at least one of the repeats comprises a 4-1BBL amino acid sequence disclosed herein.

According to specific embodiments, at least one of the repeats consists of a 4-1BBL amino acid sequence disclosed herein.

According to specific embodiments, the 4-1BBL amino acid sequence comprises three repeats of an amino acid sequence comprising SEQ ID NO: 22.

According to specific embodiments, the 4-1BBL amino acid sequence comprises three repeats of an amino acid sequence consisting of SEQ ID NO: 22.

Thus, according to specific embodiments, the 4-1BBL amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84

%, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 131.

According to specific embodiments, the 4-1BBL amino acid sequence comprises an amino acid sequence having at least 80 % identify to SEQ ID NO: 131.

According to specific embodiments, the 4-1BBL amino acid sequence comprises an amino acid sequence having at least 85 % identify to SEQ ID NO: 131.

According to specific embodiments, the 4-1BBL amino acid sequence comprises an amino acid sequence having at least 90 % identify to SEQ ID NO: 131.

According to specific embodiments, the 4-1BBL amino acid sequence comprises an amino acid sequence having at least 95 % identify to SEQ ID NO: 131.

According to specific embodiments, the 4-1BBL amino acid sequence comprises SEQ ID NO: 131.

According to specific embodiments, the 4-1BBL amino acid sequence consists of SEQ ID NO: 131.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 70 %, at least 75 %, at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 132.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 95 % identity to SEQ ID NO: 132.

According to specific embodiments, the 4-1BBL nucleic acid sequence has at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 132.

According to specific embodiments, the 4-1BBL nucleic acid sequence comprises SEQ ID NO: 132.

According to specific embodiments, the 4-1BBL nucleic acid sequence consists of SEQ ID NO: 132.

The terms “DSP” and “fusion protein”, “chimeric protein” or “chimera” are used herein interchangeably, and refer to an amino acid sequence having two or more parts which are not found together in a single amino acid sequence in nature.

The fusion protein of some embodiments of the present invention comprises a PD1 amino acid sequence and a 4-1BBL amino acid sequence (referred to herein as PD1-4-1BBL fusion protein).

According to specific embodiments, the PD1 is N-terminal to the 4-1BBL.

According to other specific embodiments, the PD1 is C-terminal to the 4-1BBL.

The PD1-4-1BBL fusion protein of some embodiments of the present invention can comprise any PD1 as defined herein; and any 4-1BBL amino acid sequence:

(aa) being 185 - 202 amino acids in length and having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, being 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and not comprising an amino acid segment G198-E205 corresponding to SEQ ID NO: 3, being 170 – 182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and not comprising an amino acid segment A1-E23 corresponding to SEQ ID NO: 3 or being 184 amino acids in length having at least 80 % identity to SEQ ID NO: 123; and/or

(bb) comprising three repeats of a 4-1BBL amino acid sequence; such as e.g. disclosed herein, each possibility represents a separate embodiment of the present invention.

The PD1-4-1BBL fusion protein of some embodiments of the present invention can comprise any PD1 amino acid sequence:

(a) being 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or being 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and not comprising an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and/or

(b) comprising a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; such as e.g. disclosed herein; and any 4-1BBL as defined herein, each possibility represents a separate embodiment of the present invention.

The PD1-4-1BBL fusion protein of some embodiments of the present invention can comprise any PD1 amino acid sequence:

(a) being 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or being 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and not comprising an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and/or

(b) comprising a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; such as e.g. disclosed herein;

and any 4-1BBL amino acid sequence:

(aa) being 185 - 202 amino acids in length and having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, being 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and not comprising an amino acid segment G198-E205 corresponding to SEQ ID NO: 3, being 170 – 182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and not comprising an amino acid segment A1-E23 corresponding to SEQ ID NO: 3 or being 184 amino acids in length having at least 80 % identity to SEQ ID NO: 123; and/or

(bb) comprising three repeats of a 4-1BBL amino acid sequence; such as e.g. disclosed herein, each possibility represents a separate embodiment of the present invention.

The PD1-4-1BBL fusion protein of some embodiments of the present invention can comprise any PD1 as defined herein; and any 4-1BBL amino acid sequence being 185 - 202 amino acids in length and having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25 such as e.g. disclosed herein, each possibility represents a separate embodiment of the present invention.

The PD1-4-1BBL fusion protein of some embodiments of the present invention can comprise any PD1 amino acid sequence:

(a) being 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2 and/or

(b) comprising a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; such as e.g. disclosed herein; and any 4-1BBL as defined herein, each possibility represents a separate embodiment of the present invention.

The PD1-4-1BBL fusion protein of some embodiments of the present invention can comprise any PD1 amino acid sequence being 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2 such as e.g. disclosed herein; and any 4-1BBL as defined herein, each possibility represents a separate embodiment of the present invention.

The PD1-4-1BBL fusion protein of some embodiments of the present invention can comprise any PD1 amino acid sequence:

57

(a) being 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2 and/or

5 (b) comprising a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; such as e.g. disclosed herein; and any 4-1BBL amino acid sequence being 185 - 202 amino acids in length and having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25 such as e.g. disclosed herein, each possibility represents a separate embodiment of the present invention.

10 The PD1-4-1BBL fusion protein of some embodiments of the present invention can comprise any PD1 amino acid sequence being 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2 such as e.g. disclosed herein; and any 4-1BBL amino acid sequence being 185 - 202 amino acids
15 in length and having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25 such as e.g. disclosed herein, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least
20 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 2, 18-21, 85, 89, 93, 27, 30 and/or 31; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the
25 present invention.

According to specific embodiments, the PD1 amino acid sequence has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO:
30 73, 75, 79, 81, 83, 87, 91 and/or 95; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 2, 18-21, 27, 30 and/or 31; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 2, 18-21, 85, 89, 93, 27, 30 or 31; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length comprising SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 73, 75, 79, 81, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length comprising SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 73; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length comprising SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length comprising SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 75, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length comprising SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 73, 75, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length comprising SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 2, 18-21, 27, 30 or 31; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length

comprising SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 2, 18-21, 85, 89, 93, 27, 30 or 31; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 73, 75, 79, 81, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 73; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 75, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 73, 75, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 2, 18-21, 27, 30 or 31; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence:

(a) is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 18-21, 85, 89 and/or 93; and/or

(b) comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and the 4-1BBL amino acid sequence has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 73, 75, 79, 81, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

60

at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 3, 22-25 and/or 36, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 18, 19, 20 and/or 21; and the 4-1BBL amino acid sequence has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 3, 22-25 and/or 36, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence:

(a) is 123 – 148 amino acids in length comprising SEQ ID NO: 18-21, 85, 89 and/or 93; and/or

(b) comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and the 4-1BBL amino acid sequence comprises SEQ ID NO: 3, 22-25 or 36, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 18-21, 85, 89 or 93; and the 4-1BBL amino acid sequence comprises SEQ ID NO: 3, 22-25 or 36, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 73, 75, 79, 81, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence comprises SEQ ID NO: 3, 22-25 or 36, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 73; and the 4-1BBL amino acid sequence comprises SEQ ID NO: 3, 22-25 or 36, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence comprises SEQ ID NO: 3, 22-25 or 36, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 75, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence comprises SEQ ID NO: 3, 22-25 or 36, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 73, 75, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence comprises SEQ ID NO: 3, 22-25 or 36, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 18, 19, 20 or 21; and the 4-1BBL amino acid sequence comprises SEQ ID NO: 3, 22-25 or 36, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 18, 19, 20 or 21; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 3, 22-25 or 36, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 18; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 18; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length and comprising SEQ ID NO: 22, 23, 24 or 25.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 18; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 18; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22, 23 or 24.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 18; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length comprising SEQ ID NO: 22, 23 or 24.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 18; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23 or 24.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 18; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 18; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length comprising SEQ ID NO: 22.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 18; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 18; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 23.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 18; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length comprising SEQ ID NO: 23.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 18; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 23.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 18; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 24.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 18; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length comprising SEQ ID NO: 24.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 18; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 24.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 19; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 19; and the 4-1BBL amino acid sequence is 185 – 202 amino acids in length comprising SEQ ID NO: 22, 23, 24 or 25.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 19; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 20; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 20; and the 4-1BBL amino acid sequence is 185 – 202 amino acids in length comprising embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 20; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %

64

or 100 % identity to SEQ ID NO: 21; and the 4-1BBL amino acid sequence is 185 – 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22, 23, 24 or 25, each possibility represents a separate embodiment of the present invention.

5 According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 21; and the 4-1BBL amino acid sequence is 185 – 202 amino acids in length comprising SEQ ID NO: 22, 23, 24 or 25.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 21; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25.

10 According to specific embodiments, the PD1 amino acid sequence:

(a) is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 18-21, 85, 89 or 93
15 and/or

(b) comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 22, each possibility represents a separate embodiment of the present invention.

20 According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 85, 89 or 93; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length and comprising SEQ ID NO: 22.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 85, 89 or 93; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24
25 or 25. According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length and comprising SEQ ID NO: 22.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is as set forth in SEQ ID
30 NO: 22, 23, 24 or 25.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 75, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length and comprising SEQ ID NO: 22.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 75, 83, 87, 91 or 95; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 75; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length and comprising SEQ ID NO: 22.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 75; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 83; and the 4-1BBL amino acid sequence is 185 - 202 amino acids in length and comprising SEQ ID NO: 22.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 783; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 24 or 25.

According to specific embodiments, the PD1 amino acid sequence comprises SEQ ID NO: 73 and the 4-1BBL amino acid sequence comprises SEQ ID NO: 3.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 73; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 3.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 115 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and the 4-1BBL amino acid sequence is any 4-1BBL as defined herein, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 115 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and the 4-1BBL amino acid sequence:

(aa) is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170 – 197 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 115 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and the 4-1BBL amino acid sequence:

at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and not comprising an amino acid segment G198-E205 corresponding to SEQ ID NO: 3, is 170 – 182 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 125 and not comprising an amino acid segment A1-E23 corresponding to SEQ ID NO: 3 or is 184 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 123; and/or

(bb) comprises three repeats of a 4-1BBL amino acid sequence, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 115 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and the 4-1BBL amino acid sequence is 170 – 182 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 125 and not comprising an amino acid segment A1-E23 corresponding to SEQ ID NO: 3.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 115; and the 4-1BBL amino acid sequence is 170 – 182 amino acids in length comprising SEQ ID NO: 125.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 115; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 125.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92

%, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 115 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and the 4-1BBL amino acid sequence is 184 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84
 5 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 123.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 115; and the 4-1BBL amino acid sequence is 184 amino acids
 10 in length comprising SEQ ID NO: 123.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 115; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 123.

According to specific embodiments, the PD1 amino acid sequence is 138-145 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85
 15 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 117 and 119 and not comprising an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and the 4-1BBL amino acid sequence is any 4-1BBL as defined herein, each possibility
 20 represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is 138-145 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85
 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99
 25 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 117 and 119 and not comprising an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and the 4-1BBL amino acid sequence:

(aa) is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170 – 197 amino acids in
 30 length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and not comprising an amino acid segment G198-E205 corresponding to SEQ ID

NO: 3, is 170 – 182 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 125 and not comprising an amino acid segment A1-E23 corresponding to SEQ ID NO: 3 or is 184 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 123; and/or

(bb) comprises three repeats of a 4-1BBL amino acid sequence, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1 amino acid sequence is 138-145 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 117 and 119 and not comprise an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and the 4-1BBL amino acid sequence is 170 – 197 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and not comprising an amino acid segment G198-E205 corresponding to SEQ ID NO: 3.

According to specific embodiments, the PD1 amino acid sequence is 138-145 amino acids in length comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 117 and 119; and the 4-1BBL amino acid sequence is 170 – 197 amino acids in length comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 117 or 119; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 127 or 129.

According to specific embodiments, the PD1 amino acid sequence is 138-145 amino acids in length comprising SEQ ID NO: 117; and the 4-1BBL amino acid sequence is 170 – 197 amino acids in length comprising SEQ ID NO: 127.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 117; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 127.

According to specific embodiments, the PD1 amino acid sequence is 138-145 amino acids in length comprising SEQ ID NO: 119; and the 4-1BBL amino acid sequence is 170 – 197 amino acids in length comprising SEQ ID NO: 129.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 119; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 129.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 115 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and the 4-1BBL amino acid sequence is 184 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 123.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length comprising SEQ ID NO: 115; and the 4-1BBL amino acid sequence is 184 amino acids in length having comprising SEQ ID NO: 123.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 115; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 123.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length or 138-145 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 18; and the 4-1BBL amino acid sequence has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 3.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length or 138-145 amino acids in length comprising SEQ ID NO: 18; and the 4-1BBL amino acid sequence comprises SEQ ID NO: 3.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 18; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 3.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length or 138-145 amino acids in length having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 18; and the 4-1BBL amino acid sequence has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 131.

According to specific embodiments, the PD1 amino acid sequence is 123 – 148 amino acids in length or 138-145 amino acids in length comprising SEQ ID NO: 18; and the 4-1BBL amino acid sequence comprises SEQ ID NO: 131.

According to specific embodiments, the PD1 amino acid sequence is as set forth in SEQ ID NO: 18; and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 131.

Non-limiting examples of specific combinations of PD1 amino acid sequence and 4-1BBL amino acid sequence which can be used with specific embodiments of the present invention are provided in Table 4 of the Examples section which follows, which serves as an integral part of the specification.

According to specific embodiments, the isolated polypeptide comprising the PD1 amino acid sequence or the isolated polypeptide comprising the 4-1BBL amino acid sequence is soluble (i.e., not immobilized to a synthetic or a naturally occurring surface).

According to specific embodiments, the isolated polypeptide comprising the PD1 amino acid sequence or the isolated polypeptide comprising the 4-1BBL amino acid sequence is immobilized to a synthetic or a naturally occurring surface.

According to specific embodiments, the PD1-4-1BBL fusion protein is soluble (i.e., not immobilized to a synthetic or a naturally occurring surface).

According to specific embodiments, the PD1-4-1BBL fusion protein is immobilized to a synthetic or a naturally occurring surface.

According to specific embodiments the PD1-4-1BBL fusion protein is in a form of at least a homo-trimer.

According to specific embodiments, at least 10 %, at least 20 %, at least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 90 %, at least 95 % of the PD1-4-

1BBL fusion protein is in a form of at least a homo-trimer, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the at least homo-trimer comprises a homo-trimer.

According to specific embodiments, the at least homo-trimer comprises a homo-tetramer.

5 According to specific embodiments, the at least homo-trimer comprises a homo-pentamer.

According to specific embodiments, the at least homo-trimer comprises a homo-hexamer.

Methods of determining trimerization are well known in the art and include, but are not limited to NATIVE-PAGE, SEC-HPLC 2D gels, gel filtration, SEC-MALS, Analytical ultracentrifugation (AUC) Mass spectrometry (MS), capillary gel electrophoresis (CGE).

10 According to specific embodiments the at least homo-trimer is at least 100 kD, at least 120 kD, at least 140 kD, at least 160 kD, at least 180 kD in molecular weight as determined by SEC-MALLS.

According to specific embodiments the at least homo-trimer is at least 100 kD in molecular weight as determined by SEC-MALS.

15 According to specific embodiments the at least homo-trimer is at least 140 kD in molecular weight as determined by SEC-MALS.

According to specific embodiments the at least homo-trimer is at least 200 kD in molecular weight as determined by SEC-MALS.

20 According to specific embodiments, the PD1-4-1BBL does not comprise a linker between the PD1 and the 4-1BBL.

In some embodiments, the PD1-4-1BBL comprises a linker which may be at any length.

Hence, according to specific embodiments the PD1-4-1BBL fusion protein comprises a linker between said PD1 and said 4-1BBL.

Any linker known in the art can be used with specific embodiments of the invention.

25 According to specific embodiments, the linker may be derived from naturally-occurring multi-domain proteins or is an empirical linker as described, for example, in Chichili et al., (2013), Protein Sci. 22(2): 153-167, Chen et al, (2013), Adv Drug Deliv Rev. 65(10): 1357-1369, the entire contents of which are hereby incorporated by reference. In some embodiments, the linker may be designed using linker designing databases and computer programs such as those described in Chen
30 et al., (2013), Adv Drug Deliv Rev. 65(10): 1357-1369 and Crasto et al., (2000), Protein Eng. 13(5):309-312, the entire contents of which are hereby incorporated by reference.

According to specific embodiments, the linker is a synthetic linker such as PEG.

According to specific embodiments, the linker is an Fc domain or the hinge region of an antibody (e.g., of IgG, IgA, IgD or IgE) or a fragment thereof.

According to specific embodiments, the linker is an Fc domain or the hinge region of human IgG4.

According to specific embodiments, the Fc domain linker comprises SEQ ID NO: 160.

According to specific embodiments, the linker is an Fc domain or the hinge region of human IgG1.

According to specific embodiments, the Fc domain linker comprises SEQ ID NO: 163.

According to specific embodiments, the Fc domain or the hinge region linker may comprise conservative and non-conservative amino acid substitutions (also referred to herein as mutations). Such substitution are known in the art.

According to other specific embodiments, the linker is not an Fc domain or a hinge region of an antibody or a fragment thereof.

According to specific embodiments, the linker may be functional. For example, without limitation, the linker may function to improve the folding and/or stability, improve the expression, improve the pharmacokinetics, and/or improve the bioactivity of the PD1-4-1BBL fusion protein.

In another example, the linker may function to target the PD1-4-1BBL fusion protein to a particular cell type or location.

According to specific embodiments, the linker is a polypeptide.

Non-limiting examples of polypeptide linkers include linkers having the sequence LE, GGGGS (SEQ ID NO: 150), (GGGGS)_n (n=1 -4) (SEQ ID NO: 149), GGGGSGGGG (SEQ ID NO: 122), (GGGGS)_{x2} (SEQ ID NO: 151), (GGGGS)_{x2}+GGGG (SEQ ID NO: 121), (Gly)₈, (Gly)₆, (EAAAK)_n (n=1 -3) (SEQ ID NO: 152), A(EAAAK)_nA (n = 2-5) (SEQ ID NO: 153), AEAAAKEAAKA (SEQ ID NO: 154), A(EAAAK)₄ALEA(EAAAK)₄A (SEQ ID NO: 155), PAPAP (SEQ ID NO: 156), K ESGSVSS EQ LAQ FRS LD (SEQ ID NO: 157), EGKSSGSGSESKST (SEQ ID NO: 158), GSAGSAAGSGEF (SEQ ID NO: 159), and (XP)_n, with X designating any amino acid, e.g., Ala, Lys, or Glu.

According to specific embodiments, the linker is selected from the group consisting of GGGGS (SEQ ID NO: 150), (GGGGS)_n (n=1 -4) (SEQ ID NO: 149), GGGGSGGGG (SEQ ID NO: 122), (GGGGS)_{x2} (SEQ ID NO: 151), (GGGGS)_{x2}+GGGG (SEQ ID NO: 121).

According to specific embodiments, the linker is a (GGGGS)_n(n=1-4) (SEQ ID NO: 149) linker.

According to specific embodiments, the linker is GGGGS_{x2} (SEQ ID NO: 151) linker.

Thus, according to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at

least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 145.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
5 comprises an amino acid sequence having at least 80 % identity to SEQ ID NO: 145.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 85 % identity to SEQ ID NO: 145.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 90 % identity to SEQ ID NO: 145.

10 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 145.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to SEQ ID NO: 145.

15 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 145.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 145.

According to specific embodiments, the linker is a GGGGSGGGG (SEQ ID NO: 122) linker.

20 According to specific embodiments, the linker is a (GGGGS)x2+GGGG (SEQ ID NO: 121) linker.

Thus, according to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at
25 least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 147.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to SEQ ID NO: 147.

30 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 85 % identity to SEQ ID NO: 147.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 90 % identity to SEQ ID NO: 147.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 147.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 96 %, at least 97 %, at least 98 %, at least 99 %
5 or 100 % identity to SEQ ID NO: 147.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 147.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 147.

10 In some embodiments, the PD1-4-1BBL fusion protein comprises a linker at a length of one to six amino acids.

According to specific embodiments, the linker is substantially comprised of glycine and/or serine residues (e.g. about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 95%, or about 97% or 100 % glycines and serines).

15 According to specific embodiments, the linker is a single amino acid linker.

In some embodiments of the invention, the one amino acid which links PD1 and 4-1BBL is glycine, also referred to herein as PD1-G-4-1BBL fusion protein.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, 20 at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

25 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group
30 consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107 and 111, each possibility represents a separate embodiment of the present invention.

5 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group
10 consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group
15 consisting of SEQ ID NO: 97, 101, 103, 105 and 107, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group
20 consisting of SEQ ID NO: 12, 14, 16 and 44-56, each possibility represents a separate embodiment of the present invention.
25

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group
30 consisting of SEQ ID NO: 12, 14 and 16, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected

from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
5 comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
10 comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
15 comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16, each possibility represents a separate embodiment of the present invention.

20 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 85 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

25 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 85 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
30 comprises an amino acid sequence having at least 85 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
comprises an amino acid sequence having at least 85 % identity to an amino acid sequence selected

from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 85 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 90 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to an amino acid sequence selected

from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 97.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 101.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 103.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 105.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 107.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 111.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 12.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 14.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 16.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 113.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 133.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 135.

5 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 137.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 139.

10 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 141.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 143.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to SEQ ID NO: 147.

15 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

20 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147, each possibility represents a separate embodiment of the present invention.

25 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111, each possibility represents a separate embodiment of the present invention.

30 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence an amino acid sequence having at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 96 %, at least 97 %, at least 98 %, at least 99 % or 100 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16, each possibility represents a separate embodiment of the present invention.

5 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135,
10 137, 139, 141, 143 and 147.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107 and 111.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
15 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107, each possibility represents a separate embodiment of the present invention.

20 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16 and 44-56.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and
25 16.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 97.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 101.

30 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 103.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 105.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 107.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 111.

5 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 12.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 14.

10 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 16.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 113.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 133.

15 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 135.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 137.

20 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 139.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 141.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 143.

25 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 147.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147.

30 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107 and 111.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
5 consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107, each possibility represents a separate embodiment of the
10 present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16 and 44-56.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
15 consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 97.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
20 consists of SEQ ID NO: 101.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 103.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 105.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
25 consists of SEQ ID NO: 107.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 111.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence
30 consists of SEQ ID NO: 12.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 14.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 16.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 113.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 133.

5 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 135.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 137.

10 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 139.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 141.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 143.

15 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 147.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence is 300 – 900 amino acids, 300 – 800 amino acids, 300 – 600 amino acids, 300 – 550 amino acids, 300 – 500 amino acids in length, each possibility represents a separate embodiment of the present
20 invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence is 300 – 750 amino acids in length.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence is 308 – 750 amino acids in length.

25 According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence is 308 – 400 amino acids in length.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence is 308 – 350 amino acids in length.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence is
30 308 – 448, 308 – 362, 308 – 351, 313 – 351, 313 – 339, 318 – 351, 318 – 362, 318 – 340, 318 – 332 amino acids in length, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence is 308 – 488 amino acids in length.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence is 308 – 351 amino acids in length.

According to specific embodiments, the PD1-4-1BBL fusion protein amino acid sequence is 318 – 351 amino acids in length.

5 Non-limiting examples of specific PD1-4-1BBL fusion proteins which can be used with specific embodiments of the present invention are provided in Table 4 of the Examples section which follows, which serves as an integral part of the specification.

According to specific embodiments, the production yield of the PD1-4-1BBL fusion protein of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold,
10 at least 3 fold, at least 5 fold higher than the production yield of a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence as set forth in SEQ ID NO: 2 and a 4-1BBL amino acid sequence as set forth in SEQ ID NO: 3, under the same production conditions, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the production yield of the PD1-4-1BBL fusion protein
15 of some embodiments of the present invention is at least 1.5 fold higher than the production yield of a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence as set forth in SEQ ID NO: 2 and a 4-1BBL amino acid sequence as set forth in SEQ ID NO: 3, under the same production conditions.

According to specific embodiments, the production yield of the PD1-4-1BBL fusion protein
20 of some embodiments of the present invention is at least 2 fold higher than the production yield of a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence as set forth in SEQ ID NO: 2 and a 4-1BBL amino acid sequence as set forth in SEQ ID NO: 3, under the same production conditions.

According to specific embodiments, the production yield of the PD1-4-1BBL fusion protein
25 of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the production yield of a SEQ ID NO: 5, under the same production conditions each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the production yield of the PD1-4-1BBL fusion protein of some embodiments of the present invention is at least 1.5 fold higher than the production yield of
30 SEQ ID NO: 5 under the same production conditions.

According to specific embodiments, the production yield of the PD1-4-1BBL fusion protein of some embodiments of the present invention is at least 2 fold higher than the production yield of a SEQ ID NO: 5 under the same production conditions.

According to specific embodiments, the production yield of the isolated polypeptide comprising the PD1 amino acid sequence of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the production yield of SEQ ID NO: 2, under the same production conditions, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the production yield of the isolated polypeptide comprising the 4-1BBL amino acid sequence of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the production yield of SEQ ID NO: 3, under the same production conditions, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the amount of aggregates of the PD1-4-1BBL fusion protein of some embodiments of the present invention is at least 20 %, at least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 90 % or at least 95 % lower than the amount of aggregates of SEQ ID NO: 5 under the same production conditions, said aggregates are of at least 300 kDa in molecular weight as may be determined by e.g. SDS-PAGE or SEC-MALS.

According to specific embodiments, the amount of aggregates of the PD1-4-1BBL fusion protein of some embodiments of the present invention is at least 20 % lower than the amount of aggregates of SEQ ID NO: 5 under the same production conditions.

According to specific embodiments, the amount of aggregates of the PD1-4-1BBL fusion protein of some embodiments of the present invention is at least 50 % lower than amount of aggregates of a SEQ ID NO: 5 under the same production conditions.

According to specific embodiments, the activity of the PD1-4-1BBL fusion protein of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the activity of a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence as set forth in SEQ ID NO: 2 and a 4-1BBL amino acid sequence as set forth in SEQ ID NO: 3, e.g. under the same production conditions, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the activity of the PD1-4-1BBL fusion protein of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the activity of a SEQ ID NO: 5, e.g. under the same production conditions, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the activity of the isolated polypeptide comprising the PD1 amino acid sequence of some embodiments of the present invention is at least 1.5 fold, at least 2

fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the activity of SEQ ID NO: 2, e.g. under the same production conditions, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the activity of the isolated polypeptide comprising the 4-
5 1BBL amino acid sequence of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the activity of SEQ ID NO: 3, e.g. under the same production conditions, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the stability of the PD1-4-1BBL fusion protein of some
10 embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the stability of a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence as set forth in SEQ ID NO: 2 and a 4-1BBL amino acid sequence as set forth in SEQ ID NO: 3, under the same production conditions, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the stability of the PD1-4-1BBL fusion protein of some
15 embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the stability of a SEQ ID NO: 5, under the same production conditions each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the stability of the isolated polypeptide comprising the
20 PD1 amino acid sequence of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the stability of SEQ ID NO: 2, under the same production conditions, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the stability of the isolated polypeptide comprising the 4-
25 1BBL amino acid sequence of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the stability of SEQ ID NO: 3, under the same production conditions, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the safety of the PD1-4-1BBL fusion protein of some
30 embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the safety of a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence as set forth in SEQ ID NO: 2 and a 4-1BBL amino acid sequence as set forth in SEQ ID NO: 3, under the same production conditions, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the safety of the PD1-4-1BBL fusion protein of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the safety of a SEQ ID NO: 5, under the same production conditions each possibility represents a separate embodiment of the present invention.

5 According to specific embodiments, the safety of the isolated polypeptide comprising the PD1 amino acid sequence of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the safety of SEQ ID NO: 2, under the same production conditions, each possibility represents a separate embodiment of the present invention.

10 According to specific embodiments, the safety of the isolated polypeptide comprising the 4-1BBL amino acid sequence of some embodiments of the present invention is at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 5 fold higher than the safety of SEQ ID NO: 3, under the same production conditions, each possibility represents a separate embodiment of the present invention.

15 According to specific embodiments, the production conditions comprise expression in a mammalian cell and culturing at 32 – 37 °C, 5 – 10 % CO₂ for 5 - 13 days.

Non-limiting examples of production conditions that can be used with specific embodiments of the invention are disclosed in the Examples section which follows.

Thus, for example an expression vector encoding the fusion protein, the isolated polypeptide
20 comprising the PD1 amino acid sequence or the isolated polypeptide comprising the 4-1BBL amino acid sequence including an artificial signal peptide (e.g. SEQ ID NO: 4) in the N terminus and His-tag and a stop codon in the C terminus, is expressed in mammalian cells such as Expi293F or ExpiCHO cells. The transduced cells are then cultured at 32 – 37°C 5 – 10 % CO₂ in cell-specific culture medium according to the Expi293F or ExpiCHO cells manufacturer instructions
25 (Thermo) and following at least 5 days in culture the proteins are collected from the supernatant and purified.

According to specific embodiments the culture is operated in a batch, split-batch, fed-batch, or perfusion mode.

According to specific embodiments, the culture is operated under fed-batch conditions.

30 According to specific embodiments, the culturing is effected at 37 °C.

According to specific embodiments, the culturing is effected at 37 °C with a temperature shift to 32 °C. This temperature shift can be effected to slow down cells metabolism prior to reaching a stationary phase.

According to specific embodiments, the isolated polypeptide comprising the PD1 amino acid sequence is capable of binding PDL1.

According to specific embodiments, the isolated polypeptide comprising the PD1 amino acid sequence of some embodiments of the present invention has an enhanced activity as disclosed
5 herein compared to SEQ ID NO: 2.

According to specific embodiments, the isolated polypeptide comprising the 4-1BBL amino acid sequence is capable of at least one of:

- (i) binding 4-1BB,
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
10 (iii) co-stimulating immune cells expressing said 4-1BB.

According to specific embodiments, the isolated polypeptide comprising the 4-1BBL amino acid sequence of some embodiments of the present invention has an enhanced activity as disclosed herein compared to SEQ ID NO: 3.

According to specific embodiments, the isolated polypeptide comprising the 4-1BBL amino
15 acid sequence is capable of (i), (ii), (iii), (i)+(ii), (i)+(iii), (ii)+(iii), (i)+(ii)+(iii).

According to specific embodiments, the PD1-4-1BBL fusion protein is capable of least one of:

- (i) binding PDL1 and 4-1BB,
- (ii) activating 4-1BB signaling pathway in an immune cell (e.g. T cell) expressing 4-1BB;
20 and/or
- (iii) activating immune cells (e.g. T cells) expressing said 4-1BB.

According to specific embodiments, the PD1-4-1BBL fusion protein of some embodiments of the present invention has an enhanced activity as disclosed herein compared to a fusion protein comprising a PD1 amino acid sequence as set forth in SEQ ID NO: 2 and a 4-1BBL amino acid
25 sequence as set forth in SEQ ID NO: 3.

According to specific embodiments, the PD1-4-1BBL fusion protein of some embodiments of the present invention has an enhanced activity as disclosed herein compared to SEQ ID NO: 5.

According to specific embodiments, the PD1-4-1BBL fusion protein is capable of (i), (ii), (iii), (i)+(ii), (i)+(iii), (ii)+(iii).

According to specific embodiments, the PD1-4-1BBL fusion protein is capable of
30 (i)+(ii)+(iii).

Methods of determining binding, activating 4-1BB signaling pathway and activating immune cells are well known in the art and are further described hereinabove and in the Examples section which follows.

As the compositions of some embodiments of present invention (e.g. the fusion protein, the isolated polypeptide comprising the PD1 amino acid sequence, the isolated polypeptide comprising the 4-1BBL amino acid sequence, a polynucleotide or nucleic acid encoding same or a host cell expressing same) are capable of activating immune cells, they can be used in method of activating immune cells, in-vitro, ex-vivo and/or in-vivo.

Thus, according to an aspect of the present invention, there is provided a method of activating immune cells, the method comprising in-vitro or ex-vivo activating immune cells in the presence of the PD1-4-1BBL fusion protein, the isolated polypeptide comprising the PD1 amino acid sequence and/or the isolated polypeptide comprising the 4-1BBL amino acid sequence disclosed herein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

According to another aspect of the present invention, there is provided a method of activating T cells, the method comprising in-vitro or ex-vivo activating T cells in the presence of the PD1-4-1BBL fusion protein the isolated polypeptide comprising the PD1 amino acid sequence and/or the isolated polypeptide comprising the 4-1BBL amino acid sequence disclosed herein and cells expressing PDL1.

According to specific embodiments, the activating is in the presence of the PD1-4-1BBL fusion protein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

According to specific embodiments, the activating is in the presence of the isolated polypeptide comprising the PD1 amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

According to specific embodiments, the activating is in the presence of the isolated polypeptide comprising the 4-1BBL amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

According to specific embodiments, the activating is in the presence of the isolated polypeptide comprising the PD1 amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same; and the 4-1BBL amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

According to specific embodiments, the immune cells express 4-1BB.

According to specific embodiments, the immune cells comprise peripheral mononuclear blood cells (PBMCs).

As used herein the term “peripheral mononuclear blood cells (PBMCs)” refers to a blood cell having a single nucleus and includes lymphocytes, monocytes and dendritic cells (DCs).

According to specific embodiments, the PBMCs are selected from the group consisting of dendritic cells (DCs), T cells, B cells, NK cells and NKT cells.

5 According to specific embodiments, the PBMCs comprise T cells, B cells, NK cells and NKT cells.

Methods of obtaining PBMCs are well known in the art, such as drawing whole blood from a subject and collection in a container containing an anti-coagulant (e.g. heparin or citrate); and apheresis. Following, according to specific embodiments, at least one type of PBMCs is purified
10 from the peripheral blood. There are several methods and reagents known to those skilled in the art for purifying PBMCs from whole blood such as leukapheresis, sedimentation, density gradient centrifugation (e.g. ficoll), centrifugal elutriation, fractionation, chemical lysis of e.g. red blood cells (e.g. by ACK), selection of specific cell types using cell surface markers (using e.g. FACS
15 sorter or magnetic cell separation techniques such as are commercially available e.g. from Invitrogen, Stemcell Technologies, Cellpro, Advanced Magnetix, or Miltenyi Biotec.), and depletion of specific cell types by methods such as eradication (e.g. killing) with specific antibodies or by affinity based purification based on negative selection (using e.g. magnetic cell separation techniques, FACS sorter and/or capture ELISA labeling). Such methods are described for example in THE HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, Volumes 1 to 4,
20 (D.N. Weir, editor) and FLOW CYTOMETRY AND CELL SORTING (A. Radbruch, editor, Springer Verlag, 2000).

According to specific embodiments, the immune cells comprise tumor infiltrating lymphocytes.

As used herein the term “tumor infiltrating lymphocytes (TILs)” refers to mononuclear white
25 blood cells that have left the bloodstream and migrated into a tumor.

According to specific embodiments, the TILs are selected from the group consisting of T cells, B cells, NK cells and monocytes.

Methods of obtaining TILs are well known in the art, such as obtaining tumor samples from a subject by e.g. biopsy or necropsy and preparing a single cell suspension thereof. The single cell
30 suspension can be obtained in any suitable manner, e.g., mechanically (disaggregating the tumor using, e.g., a GentleMACS(TM) Dissociator, Miltenyi Biotec, Auburn, Calif.) or enzymatically (e.g., collagenase or DNase). Following, the at least one type of TILs can be purified from the cell suspension. There are several methods and reagents known to those skilled in the art for purifying the desired type of TILs, such as selection of specific cell types using cell surface markers (using

e.g. FACS sorter or magnetic cell separation techniques such as are commercially available e.g. from Invitrogen, Stemcell Technologies, Cellpro, Advanced Magnetix, or Miltenyi Biotec.), and depletion of specific cell types by methods such as eradication (e.g. killing) with specific antibodies or by affinity based purification based on negative selection (using e.g. magnetic cell separation techniques, FACS sorter and/or capture ELISA labeling). Such methods are described for example in THE HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, Volumes 1 to 4, (D.N. Weir, editor) and FLOW CYTOMETRY AND CELL SORTING (A. Radbruch, editor, Springer Verlag, 2000).

According to specific embodiments, the immune cells comprise phagocytic cells.

As used herein, the term “phagocytic cells” refer to a cell that is capable of phagocytosis and include both professional and non-professional phagocytic cells. Methods of analyzing phagocytosis are well known in the art and include for examples killing assays, flow cytometry and/or microscopic evaluation (live cell imaging, fluorescence microscopy, confocal microscopy, electron microscopy). According to specific embodiments, the phagocytic cells are selected from the group consisting of monocytes, dendritic cells (DCs) and granulocytes.

According to specific embodiments, the immune cells comprise monocytes.

According to specific embodiments, the term “monocytes” refers to both circulating monocytes and to macrophages (also referred to as mononuclear phagocytes) present in a tissue.

According to specific embodiments, the monocytes comprise macrophages. Typically, cell surface phenotype of macrophages include CD14, CD40, CD11b, CD64, F4/80 (mice)/EMR1 (human), lysozyme M, MAC-1/MAC-3 and CD68.

According to specific embodiments, the monocytes comprise circulating monocytes. Typically, cell surface phenotypes of circulating monocytes include CD14 and CD16 (e.g. CD14⁺⁺ CD16⁻, CD14⁺CD16⁺⁺, CD14⁺⁺CD16⁺).

According to specific embodiments, the immune cells comprise DCs.

As used herein the term “dendritic cells (DCs)” refers to any member of a diverse population of morphologically similar cell types found in lymphoid or non-lymphoid tissues. DCs are a class of professional antigen presenting cells, and have a high capacity for sensitizing HLA-restricted T cells. DCs include, for example, plasmacytoid dendritic cells, myeloid dendritic cells (including immature and mature dendritic cells), Langerhans cells, interdigitating cells, follicular dendritic cells. Dendritic cells may be recognized by function, or by phenotype, particularly by cell surface phenotype. These cells are characterized by their distinctive morphology having veil-like projections on the cell surface, intermediate to high levels of surface HLA-class II expression and ability to present antigen to T cells, particularly to naive T cells (See Steinman R, et al., Ann. Rev.

Immunol. 1991; 9:271-196.). Typically, cell surface phenotype of DCs include CD1a+, CD4+, CD86+, or HLA-DR. The term DCs encompasses both immature and mature DCs.

According to specific embodiments, the immune cells comprise granulocytes.

As used herein, the term “granulocytes” refer to polymorphonuclear leukocytes characterized by the presence of granules in their cytoplasm.

According to specific embodiments, the granulocytes comprise neutrophils.

According to specific embodiments, the granulocytes comprise mast-cells.

According to specific embodiments the immune cells comprise T cells.

As used herein, the term “T cells” refers to a differentiated lymphocyte with a CD3+, T cell receptor (TCR)+ having either CD4+ or CD8+ phenotype. The T cell may be either an effector or a regulatory T cell.

As used herein, the term “effector T cells” refers to a T cell that activates or directs other immune cells e.g. by producing cytokines or has a cytotoxic activity e.g., CD4+, Th1/Th2, CD8+ cytotoxic T lymphocyte.

As used herein, the term “regulatory T cell” or “Treg” refers to a T cell that negatively regulates the activation of other T cells, including effector T cells, as well as innate immune system cells. Treg cells are characterized by sustained suppression of effector T cell responses. According to a specific embodiment, the Treg is a CD4+CD25+Foxp3+ T cell.

According to specific embodiments, the T cells are CD4+ T cells.

According to other specific embodiments, the T cells are CD8+ T cells.

According to specific embodiments, the T cells are memory T cells. Non-limiting examples of memory T cells include effector memory CD4+ T cells with a CD3+/CD4+/CD45RA-/CCR7- phenotype, central memory CD4+ T cells with a CD3+/CD4+/CD45RA-/CCR7+ phenotype, effector memory CD8+ T cells with a CD3+/CD8+ CD45RA-/CCR7-phenotype and central memory CD8+ T cells with a CD3+/CD8+ CD45RA-/CCR7+ phenotype.

According to specific embodiments, the T cells comprise engineered T cells transduced with a nucleic acid sequence encoding an expression product of interest.

According to specific embodiments, the expression product of interest is a T cell receptor (TCR) or a chimeric antigen receptor (CAR).

As used herein the phrase “transduced with a nucleic acid sequence encoding a TCR” or “transducing with a nucleic acid sequence encoding a TCR” refers to cloning of variable α - and β -chains from T cells with specificity against a desired antigen presented in the context of MHC. Methods of transducing with a TCR are known in the art and are disclosed e.g. in Nicholson et al.

Adv Hematol. 2012; 2012:404081; Wang and Rivière Cancer Gene Ther. 2015 Mar;22(2):85-94); and Lamers et al, Cancer Gene Therapy (2002) 9, 613–623.

As used herein, the phrase "transduced with a nucleic acid sequence encoding a CAR" or "transducing with a nucleic acid sequence encoding a CAR" refers to cloning of a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen
5 recognition moiety and a T-cell activation moiety. A chimeric antigen receptor (CAR) is an artificially constructed hybrid protein or polypeptide containing an antigen binding domain of an antibody (e.g., a single chain variable fragment (scFv)) linked to T-cell signaling or T-cell activation domains. Method of transducing with a CAR are known in the art and are disclosed e.g.
10 in Davila et al. Oncoimmunology. 2012 Dec 1;1(9):1577-1583; Wang and Rivière Cancer Gene Ther. 2015 Mar;22(2):85-94); Maus et al. Blood. 2014 Apr 24;123(17):2625-35; Porter DL The New England journal of medicine. 2011, 365(8):725-733; Jackson HJ, Nat Rev Clin Oncol. 2016;13(6):370-383; and Globerson-Levin et al. Mol Ther. 2014;22(5):1029-1038.

According to specific embodiments, the immune cells comprise B cells.

15 As used herein the term "B cells" refers to a lymphocyte with a B cell receptor (BCR)+, CD19+ and or B220+ phenotype. B cells are characterized by their ability to bind a specific antigen and elicit a humoral response.

According to specific embodiments, the immune cells comprise NK cells.

As used herein the term "NK cells" refers to differentiated lymphocytes with a CD16+ CD56+
20 and/or CD57+ TCR- phenotype. NK are characterized by their ability to bind to and kill cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic enzymes, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response.

25 According to specific embodiments, the immune cells comprise NKT cells.

As used herein the term "NKT cells" refers to a specialized population of T cells that express a semi-invariant $\alpha\beta$ T-cell receptor, but also express a variety of molecular markers that are typically associated with NK cells, such as NK1.1. NKT cells include NK1.1+ and NK1.1-, as well as CD4+, CD4-, CD8+ and CD8- cells. The TCR on NKT cells is unique in that it recognizes
30 glycolipid antigens presented by the MHC I-like molecule CD1d. NKT cells can have either protective or deleterious effects due to their abilities to produce cytokines that promote either inflammation or immune tolerance.

According to specific embodiments, the immune cells are obtained from a healthy subject.

According to specific embodiments, the immune cells are obtained from a subject suffering from a pathology.

According to specific embodiments, the activating is in the presence of cells expressing PDL1 or exogenous PDL1.

5 According to specific embodiments, the activating is in the presence of exogenous PDL1, According to specific embodiments, the exogenous PDL1 is soluble.

According to other specific embodiments, the exogenous PDL1 is immobilized to a solid support.

According to specific embodiments, the activating is in the presence of cells expressing PDL1.

10 According to specific embodiments, the cells expressing the PDL1 comprise pathologic (diseased) cells.

According to specific embodiments, the cells expressing the PDL1 comprise cancer cells.

According to specific embodiments, the activating is in the presence of a stimulatory agent capable of at least transmitting a primary activating signal [e.g. ligation of the T-Cell Receptor (TCR) with the Major Histocompatibility Complex (MHC)/peptide complex on the Antigen Presenting Cell (APC)] resulting in cellular proliferation, maturation, cytokine production, phagocytosis and/or induction of regulatory or effector functions of the immune cell. According to specific embodiments, the stimulator agent can also transmit a secondary co-stimulatory signal.

Methods of determining the amount of the stimulatory agent and the ratio between the stimulatory agent and the immune cells are well within the capabilities of the skilled in the art and thus are not specified herein.

The stimulatory agent can activate the immune cells in an antigen-dependent or -independent (i.e. polyclonal) manner.

25 According to specific embodiments, stimulatory agent comprises an antigen non-specific stimulator.

Non-specific stimulators are known to the skilled in the art. Thus, as a non-limiting example, when the immune cells comprise T cells, antigen non-specific stimulator can be an agent capable of binding to a T cell surface structure and induce the polyclonal stimulation of the T cell, such as but not limited to anti-CD3 antibody in combination with a co-stimulatory protein such as anti-CD28 antibody. Other non-limiting examples include anti-CD2, anti-CD137, anti-CD134, Notch-ligands, e.g. Delta-like 1/4, Jagged1/2 either alone or in various combinations with anti-CD3. Other agents that can induce polyclonal stimulation of T cells include, but not limited to mitogens, PHA, PMA-ionomycin, CEB and CytoStim (Miltenyi Biotech). According to specific embodiments, the antigen non-specific stimulator comprises anti-CD3 and anti-CD28 antibodies. According to

specific embodiments, the T cell stimulator comprises anti-CD3 and anti-CD28 coated beads, such as the CD3CD28 MACSiBeads obtained from Miltenyi Biotec.

According to specific embodiments, the stimulatory agent comprises an antigen-specific stimulator.

5 Non-limiting examples of antigen specific T cell stimulators include an antigen-loaded antigen presenting cell [APC, e.g. dendritic cell] and peptide loaded recombinant MHC. Thus, for example, a T cells stimulator can be a dendritic cell preloaded with a desired antigen (e.g. a tumor antigen) or transfected with mRNA coding for the desired antigen.

According to specific embodiments, the antigen is a cancer antigen.

10 As used herein, the term “cancer antigen” refers to an antigen overexpressed or solely expressed by a cancerous cell as compared to a non-cancerous cell. A cancer antigen may be a known cancer antigen or a new specific antigen that develops in a cancer cell (i.e. neoantigens).

Non-limiting examples for known cancer antigens include MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, 15 GAGE-8, BAGE-1, RAGE-1, LB33/MUM-1, PRAME, NAG, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1/CT7, MAGE-C2, NY-ES0-1, LAGE-1, SSX-1, SSX-2(HOM-MEL-40), SSX-3, SSX-4, SSX-5, SCP-1 and XAGE, melanocyte differentiation antigens, p53, ras, CEA, MUC1, PMSA, PSA, tyrosinase, Melan-A, MART-1, 20 gp100, gp75, alphaactinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAA0205, Mart2, Mum-2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR alpha fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomerase, GnTV, Herv-K-mel, NA-88, SP17, and TRP2-Int2, (MART-1), E2A-PRL, H4-RET, IGH-IGK, 25 MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p185erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, alpha-fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, C0-029, FGF-5, 0250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\170K, 30 NYCO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, tyrosinase related proteins, TRP-1, or TRP-2.

Other tumor antigens that may be expressed are well-known in the art (see for example W000/20581; Cancer Vaccines and Immunotherapy (2000) Eds Stern, Beverley and Carroll, Cambridge University Press, Cambridge). The sequences of these tumor antigens are readily

available from public databases but are also found in WO 1992/020356 AI, WO 1994/005304 AI, WO 1994/023031 AI, WO 1995/020974 AI, WO 1995/023874 AI & WO 1996/026214 AI.

Alternatively, or additionally, a tumor antigen may be identified using cancer cells obtained from the subject by e.g. biopsy.

5 Thus, according to specific embodiments, the stimulatory agent comprises a cancer cell.

According to specific embodiments, the activating is in the presence of an anti-cancer agent.

According to specific embodiments, the immune cells are purified following the activation.

Thus, the present invention also contemplated isolated immune cells obtainable according to the methods of the present invention.

10 According to specific embodiments, the immune cells used and/or obtained according to the present invention can be freshly isolated, stored e.g., cryopreserved (i.e. frozen) at e.g. liquid nitrogen temperature at any stage for long periods of time (e.g., months, years) for future use; and cell lines.

Methods of cryopreservation are commonly known by one of ordinary skill in the art and are disclosed e.g. in International Patent Application Publication Nos. WO2007054160 and WO
15 2001039594 and US Patent Application Publication No. US20120149108.

According to specific embodiments, the cells obtained according to the present invention can be stored in a cell bank or a depository or storage facility.

Consequently, the present teachings further suggest the use of the isolated immune cells and
20 the methods of the present invention as, but not limited to, a source for adoptive immune cells therapies for diseases that can benefit from activating immune cells e.g. a hyper-proliferative disease; a disease associated with immune suppression and infections.

Thus, according to specific embodiments, method of the present invention comprises adoptively transferring the immune cells following said activating to a subject in need thereof.

25 According to specific embodiments, there is provided the immune cells obtainable according to the methods of the present invention for use in adoptive cell therapy.

The cells used according to specific embodiments of the present invention may be autologous or non-autologous; they can be syngeneic or non-syngeneic: allogeneic or xenogeneic to the subject; each possibility represents a separate embodiment of the present invention.

30 The present teachings also contemplate the use of the compositions of the present invention (e.g. the fusion protein, the isolated polypeptide comprising the PD1 amino acid sequence, the isolated polypeptide comprising the 4-1BBL amino acid sequence, a polynucleotide or nucleic acid construct encoding same or a host cell expressing same) in methods of treating a disease that can benefit from activating immune cells.

Thus, according to another aspect of the present invention, there is provided a method of treating a disease that can benefit from activating immune cells comprising administering to a subject in need thereof the PD1-4-1BBL fusion protein, the isolated polypeptide comprising the PD1 amino acid sequence and/or the isolated polypeptide comprising the 4-1BBL amino acid sequence disclosed herein, a polynucleotide or nucleic acid construct encoding same or a host cell encoding same.

According to another aspect of the present invention, there is provided the PD1-4-1BBL fusion protein, the isolated polypeptide comprising the PD1 amino acid sequence and/or the isolated polypeptide comprising the 4-1BBL amino acid sequence disclosed herein, a polynucleotide or nucleic acid construct encoding same or a host cell encoding same for use in the treatment of a disease that can benefit from activating immune cells.

According to specific embodiments, the treating or the treatment is with the PD1-4-1BBL fusion protein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

According to specific embodiments, the treating or the treatment is with the isolated polypeptide comprising the PD1 amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

According to specific embodiments, the treating or the treatment is with the isolated polypeptide comprising the 4-1BBL amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

According to specific embodiments, the treating or the treatment is with the isolated polypeptide comprising the PD1 amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same; and the 4-1BBL amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

The term “treating” or “treatment” refers to inhibiting, preventing or arresting the development of a pathology (disease, disorder or medical condition) and/or causing the reduction, remission, or regression of a pathology or a symptom of a pathology. Those of skill in the art will understand that various methodologies and assays can be used to assess the development of a pathology, and similarly, various methodologies and assays may be used to assess the reduction, remission or regression of a pathology.

As used herein, the term “subject” includes mammals, e.g., human beings at any age and of any gender. According to specific embodiments, the term “subject” refers to a subject who suffers

from the pathology (disease, disorder or medical condition). According to specific embodiments, this term encompasses individuals who are at risk to develop the pathology.

According to specific embodiments, the subject is afflicted with a disease associated with cells expressing PDL1.

5 According to specific embodiments, diseased cells of the subject express PDL1.

As used herein the phrase “a disease that can benefit from activating immune cells” refers to diseases in which the subject’s immune response activity may be sufficient to at least ameliorate symptoms of the disease or delay onset of symptoms, however for any reason the activity of the subject’s immune response in doing so is less than optimal.

10 Non-limiting examples of diseases that can benefit from activating immune cells include hyper-proliferative diseases, diseases associated with immune suppression, immunosuppression caused by medication (e.g. mTOR inhibitors, calcineurin inhibitor, steroids) and infections.

According to specific embodiments, the disease comprises a hyper-proliferative disease.

According to specific embodiments, the hyper-proliferative disease comprises sclerosis, fibrosis, 15 Idiopathic pulmonary fibrosis, psoriasis, systemic sclerosis/scleroderma, primary biliary cholangitis, primary sclerosing cholangitis, liver fibrosis, prevention of radiation-induced pulmonary fibrosis, myelofibrosis or retroperitoneal fibrosis.

According to other specific embodiments, the hyper-proliferative disease comprises cancer.

Thus, according to another aspect of the present invention, there is provided a method of 20 treating cancer comprising administering the PD1-4-1BBL fusion protein, the isolated polypeptide comprising the PD1 amino acid sequence and/or the isolated polypeptide comprising the 4-1BBL amino acid sequence disclosed herein to a subject in need thereof.

As used herein, the term cancer encompasses both malignant and pre-malignant cancers.

With regard to pre-malignant or benign forms of cancer, optionally the compositions and 25 methods thereof may be applied for halting the progression of the pre-malignant cancer to a malignant form.

Cancers which can be treated by the methods of some embodiments of the invention can be any solid or non-solid cancer and/or cancer metastasis.

According to specific embodiments, the cancer comprises malignant cancer.

30 Cancers which can be treated by the methods of some embodiments of the invention can be any solid or non-solid cancer and/or cancer metastasis. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, lung cancer (including small-cell lung cancer, non-small-cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer

of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as
 5 B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; Burkitt lymphoma, Diffused large B cell lymphoma (DLBCL), high grade lymphoblastic NHL; high-grade small non-cleaved cell NHL; bulky disease NHL; mantle
 10 cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); T cell lymphoma, Hodgkin lymphoma, chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Acute myeloid leukemia (AML), Acute promyelocytic leukemia (APL), Hairy cell leukemia; chronic myeloblastic leukemia (CML); and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema
 15 (such as that associated with brain tumors), and Meigs' syndrome. Preferably, the cancer is selected from the group consisting of breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, melanoma, ovarian cancer, mesothelioma, and multiple myeloma. The cancerous
 20 conditions amenable for treatment of the invention include metastatic cancers.

According to specific embodiments, the cancer comprises pre-malignant cancer.

Pre-malignant cancers (or pre-cancers) are well characterized and known in the art (refer, for example, to Berman JJ. and Henson DE., 2003. Classifying the precancers: a metadata approach. BMC Med Inform Decis Mak. 3:8). Classes of pre-malignant cancers amenable to treatment via
 25 the method of the invention include acquired small or microscopic pre-malignant cancers, acquired large lesions with nuclear atypia, precursor lesions occurring with inherited hyperplastic syndromes that progress to cancer, and acquired diffuse hyperplasias and diffuse metaplasias. Examples of small or microscopic pre-malignant cancers include HGSIL (High grade squamous intraepithelial lesion of uterine cervix), AIN (anal intraepithelial neoplasia), dysplasia of vocal
 30 cord, aberrant crypts (of colon), PIN (prostatic intraepithelial neoplasia). Examples of acquired large lesions with nuclear atypia include tubular adenoma, AILD (angioimmunoblastic lymphadenopathy with dysproteinemia), atypical meningioma, gastric polyp, large plaque parapsoriasis, myelodysplasia, papillary transitional cell carcinoma in-situ, refractory anemia with excess blasts, and Schneiderian papilloma. Examples of precursor lesions occurring with inherited

hyperplastic syndromes that progress to cancer include atypical mole syndrome, C cell adenomatosis and MEA. Examples of acquired diffuse hyperplasias and diffuse metaplasias include AIDS, atypical lymphoid hyperplasia, Paget's disease of bone, post-transplant lymphoproliferative disease and ulcerative colitis.

5 According to specific embodiments, the cancer is Acute Myeloid Leukemia, Anal Cancer, Basal Cell Carcinoma, B-Cell Non-Hodgkin Lymphoma, Bile Duct Cancer, Bladder Cancer, Breast Cancer, Cervical Cancer, Chronic Lymphocytic Leukemia (CLL), Chronic Myelocytic Leukemia (CML), Colorectal Cancer, Cutaneous T-Cell Lymphoma, Diffuse Large B-Cell Lymphoma, Endometrial Cancer, Esophageal Cancer, Fallopian Tube Cancer, Follicular
10 Lymphoma, Gastric Cancer, Gastroesophageal (GE) Junction Carcinomas, Germ Cell Tumors, Germinomatous (Seminomatous), Germ Cell Tumors, Glioblastoma Multiforme (GBM), Gliosarcoma, Head And Neck Cancer, Hepatocellular Carcinoma, Hodgkin Lymphoma, Hypopharyngeal Cancer, Laryngeal Cancer, Leiomyosarcoma, Mantle Cell Lymphoma, Melanoma, Merkel Cell Carcinoma, Multiple Myeloma, Neuroendocrine Tumors, Non-Hodgkin
15 Lymphoma, Non-Small Cell Lung Cancer, Oral Cavity (Mouth) Cancer, Oropharyngeal Cancer, Osteosarcoma, Ovarian Cancer, Pancreatic Cancer, Peripheral Nerve Sheath Tumor (Neurofibrosarcoma), Peripheral T-Cell Lymphomas (PTCL), Peritoneal Cancer, Prostate Cancer, Renal Cell Carcinoma, Salivary Gland Cancer, Skin Cancer, Small-Cell Lung Cancer, Soft Tissue Sarcoma, Squamous Cell Carcinoma, Synovial Sarcoma, Testicular Cancer, Thymic Carcinoma,
20 Thyroid Cancer, Ureter Cancer, Urethral Cancer, Uterine Cancer, Vaginal Cancer or Vulvar Cancer.

According to specific embodiments, the cancer is Acute myeloid leukemia, Bladder Cancer, Breast Cancer, chronic lymphocytic leukemia, Chronic myelogenous leukemia, Colorectal cancer, Diffuse large B-cell lymphoma, Epithelial Ovarian Cancer, Epithelial Tumor, Fallopian Tube
25 Cancer, Follicular Lymphoma, Glioblastoma multiform, Hepatocellular carcinoma, Head and Neck Cancer, Leukemia, Lymphoma, Mantle Cell Lymphoma, Melanoma, Mesothelioma, Multiple Myeloma, Nasopharyngeal Cancer, Non Hodgkin lymphoma, Non-small-cell lung carcinoma, Ovarian Cancer, Prostate Cancer or Renal cell carcinoma.

According to specific embodiments, the cancer is selected from the group consisting of
30 lymphoma, leukemia and carcinoma.

According to specific embodiments, the cancer is selected from the group consisting of lymphoma, leukemia, colon cancer, pancreatic cancer, ovarian cancer, lung cancer and squamous cell carcinoma.

According to specific embodiments, the cancer is colon carcinoma.

According to specific embodiments, the cancer is ovarian carcinoma.

According to specific embodiments, the cancer is lung carcinoma.

According to specific embodiments, the cancer is head and neck carcinoma.

According to specific embodiments, the cancer is leukemia.

5 According to specific embodiments, the leukemia is selected from the group consisting of acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophilic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, (Oss' 10 leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, 15 myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, plasmacytic leukemia, promyelocytic leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, and undifferentiated cell leukemia.

According to specific embodiments, the leukemia is promyelocytic leukemia, acute myeloid leukemia or chronic myelogenous leukemia.

20 According to specific embodiments, the cancer is lymphoma.

According to specific embodiments, the lymphoma is B cell lymphoma

According to specific embodiments, the lymphoma is T cell lymphoma.

According to other specific embodiments, the lymphoma is Hodgkins lymphoma.

According to specific embodiments, the lymphoma is non-Hodgkins lymphoma.

25 According to specific embodiments, the non-Hodgkin's Lymphoma is a selected from the group consisting of aggressive NHL, transformed NHL, indolent NHL, relapsed NHL, refractory NHL, low grade non-Hodgkin's Lymphoma, follicular lymphoma, large cell lymphoma, B-cell lymphoma, T-cell lymphoma, Mantle cell lymphoma, Burkitt's lymphoma, NK cell lymphoma, diffuse large B—cell lymphoma, acute lymphoblastic lymphoma, and cutaneous T cell cancer, 30 including mycosis fungoides/Sezary syndrome.

According to specific embodiments, the cancer is multiple myeloma.

According to at least some embodiments, the multiple myeloma is selected from the group consisting of multiple myeloma cancers which produce light chains of kappa-type and/or light chains of lambda-type; aggressive multiple myeloma, including primary plasma cell leukemia

(PCL); benign plasma cell disorders such as MGUS (monoclonal gammopathy of undetermined significance), Waldenstrom's macroglobulinemia (WM, also known as lymphoplasmacytic lymphoma) which may proceed to multiple myeloma; smoldering multiple myeloma (SMM), indolent multiple myeloma, premalignant forms of multiple myeloma which may also proceed to multiple myeloma; primary amyloidosis.

According to specific embodiments, the cancer is defined by the presence of tumors that have tumor-infiltrating lymphocytes (TILs) in the tumor micro-environment and/or tumors with a relatively high expression of PDL1 in the tumor micro-environment.

According to specific embodiments, cells of the cancer express PDL1.

According to specific embodiments, the disease comprises a disease associated with immune suppression or immunosuppression caused by medication (e.g. mTOR inhibitors, calcineurin inhibitor, steroids).

According to specific embodiments, the disease comprises HIV, Measles, influenza, LCCM, RSV, Human Rhinoviruses, EBV, CMV or Parvo viruses.

According to specific embodiments, the disease comprises an infection.

As used herein, the term "infection" or "infectious disease" refers to a disease induced by a pathogen. Specific examples of pathogens include, viral pathogens, bacterial pathogens e.g., intracellular mycobacterial pathogens (such as, for example, *Mycobacterium tuberculosis*), intracellular bacterial pathogens (such as, for example, *Listeria monocytogenes*), or intracellular protozoan pathogens (such as, for example, *Leishmania* and *Trypanosoma*).

Specific types of viral pathogens causing infectious diseases treatable according to the teachings of the present invention include, but are not limited to, retroviruses, circoviruses, parvoviruses, papovaviruses, adenoviruses, herpesviruses, iridoviruses, poxviruses, hepadnaviruses, picornaviruses, caliciviruses, togaviruses, flaviviruses, reoviruses, orthomyxoviruses, paramyxoviruses, rhabdoviruses, bunyaviruses, coronaviruses, arenaviruses, and filoviruses.

Specific examples of viral infections which may be treated according to the teachings of the present invention include, but are not limited to, human immunodeficiency virus (HIV)-induced acquired immunodeficiency syndrome (AIDS), influenza, rhinoviral infection, viral meningitis, Epstein-Barr virus (EBV) infection, hepatitis A, B or C virus infection, measles, papilloma virus infection/warts, cytomegalovirus (CMV) infection, Herpes simplex virus infection, yellow fever, Ebola virus infection, rabies, etc.

According to specific embodiments, the compositions disclosed herein (e.g. PD1-4-1BBL fusion protein, polypeptide comprising a PD1 amino acid sequence, polypeptide comprising a 4-

1BBL amino acid sequence, polynucleotide or nucleic acid construct encoding same and/or host-cell expressing same) can be administered to a subject in combination with other established or experimental therapeutic regimen to treat a disease that can benefit from activating immune cells (e.g. cancer) including, but not limited to analgesics, chemotherapeutic agents, radiotherapeutic agents, cytotoxic therapies (conditioning), hormonal therapy, antibodies and other treatment regimens (e.g., surgery) which are well known in the art.

According to specific embodiments, the compositions disclosed herein (e.g. PD1-4-1BBL fusion protein, polypeptide comprising a PD1 amino acid sequence, polypeptide comprising a 4-1BBL amino acid sequence, polynucleotide or nucleic acid construct encoding same and/or host-cell expressing same) can be administered to a subject in combination with adoptive cell transplantation such as, but not limited to transplantation of bone marrow cells, hematopoietic stem cells, PBMCs, cord blood stem cells and/or induced pluripotent stem cells.

According to specific embodiments, the therapeutic agent administered in combination with the composition of some embodiments of the invention comprises an anti-cancer agent.

Thus, according to another aspect of the present invention, there is provided a method of treating cancer comprising administering to a subject in need thereof an anti-cancer agent; and the PD1-4-1BBL fusion protein, the polypeptide comprising the PD1 amino acid sequence and/or the polypeptide comprising the 4-1BBL amino acid sequence disclosed herein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

Anti-cancer agent that can be use with specific embodiments of the invention include, but are not limited to the anti-cancer drugs Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adriamycin; Adozelesin; Aldesleukin; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrone Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropiramine; Busulfan; Cactinomycin; Calusterone; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; Dactinomycin; Daunorubicin Hydrochloride; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflornithine Hydrochloride; Elsamitrucin; Enloplatin; Enpromate; Epiropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine

Phosphate; Fluorouracil; Flurocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofoosine; Interferon Alfa-2a; Interferon Alfa-2b; Interferon Alfa-n1; Interferon Alfa-n3; Interferon Beta- I a; Interferon Gamma- I b; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; 5 Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocil; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine; Meturedapa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; 10 Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; Peplomycin Sulfate; Perfosfamide; Pipobroman; Pipsulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Riboprime; Rogletimide; Safingol; Safingol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; 15 Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Sulofenur; Talisomycin; Taxol; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Tiazofuirin; Tirapazamine; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole 20 Hydrochloride; Uracil Mustard; Uredapa; Vapreotide; Verteporfin; Vinblastine Sulfate; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinatate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride. Additional antineoplastic agents include those disclosed in Chapter 52, Antineoplastic Agents (Paul Calabresi and Bruce A. Chabner), and the 25 introduction thereto, 1202-1263, of Goodman and Gilman's "The Pharmacological Basis of Therapeutics", Eighth Edition, 1990, McGraw-Hill, Inc. (Health Professions Division).

According to specific embodiments, the anti-cancer agent comprises an antibody.

According to specific embodiments, the antibody is selected from the group consisting rituximab, cetuximab, trastuzumab, edrecolomab, alemtuzumab, gemtuzumab, ibritumomab, 30 panitumumab Belimumab, Bevacizumab, Bivatuzumab mertansine, Blinatumomab, Blontuvetmab, Brentuximab vedotin, Catumaxomab, Cixutumumab, Daclizumab, Adalimumab, Bezlotoxumab, Certolizumab pegol, Citatuzumab bogatox, Daratumumab, Dinutuximab, Elotuzumab, Ertumaxomab, Etaracizumab, Gemtuzumab ozogamicin, Girentuximab, Necitumumab, Obinutuzumab, Ofatumumab, Pertuzumab, Ramucirumab, Siltuximab,

Tositumomab, Nivolumab, Pembrolizumab, Durvalumab, Atezolizumab, Avelumab Trastuzumab and ipilimumab.

According to specific embodiments, the antibody is selected from the group consisting of rituximab and cetuximab.

5 According to specific embodiments, the therapeutic agent or the anti-cancer agent comprises an IMiD (e.g. Thalidomide, Lenalidomide, Pomalidomide).

According to specific embodiments, the IMiD is selected from the group consisting of Thalidomide, Lenalidomide and Pomalidomide.

10 According to specific embodiments, the therapeutic agent administered in combination with the composition of some embodiments of the invention comprises an anti-infection agent (e.g. antibiotics and anti-viral agents)

According to specific embodiments, the therapeutic agent administered in combination with the composition of some embodiments of the invention comprises an immune suppressor agent (e.g. GCSF and other bone marrow stimulators, steroids).

15 According to specific embodiments the combination therapy has an additive effect.

According to specific embodiments, the combination therapy has a synergistic effect.

According to another aspect of the present invention there is provided an article of manufacture comprising a packaging material packaging a therapeutic agent for treating a disease that can benefit from activating immune cell; and the PD1-4-1BBL fusion protein, the polypeptide
20 comprising the PD1 amino acid sequence and/or the polypeptide comprising the 4-1BBL amino acid sequence disclosed herein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

According to specific embodiments, the article of manufacture is identified for the treatment of a disease that can benefit from activating immune cells.

25 According to specific embodiments, the therapeutic agent for treating said disease; and the PD1-4-1BBL fusion protein, the polypeptide comprising the PD1 amino acid sequence and/or the polypeptide comprising the 4-1BBL amino acid sequence, the polynucleotide encoding same, the nucleic acid construct encoding same or the host cell expressing same are packaged in separate containers.

30 According to specific embodiments, the therapeutic agent for treating said disease; and the PD1-4-1BBL fusion protein, the polypeptide comprising the PD1 amino acid sequence and/or the polypeptide comprising the 4-1BBL amino acid sequence, the polynucleotide or the nucleic acid encoding same, the nucleic acid construct encoding same or the host cell expressing same are packaged in a co-formulation.

According to specific embodiments, the article of manufacture comprises the PD1-4-1BBL fusion protein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

5 According to specific embodiments, the article of manufacture comprises the isolated polypeptide comprising the PD1 amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

According to specific embodiments, the article of manufacture comprises the isolated polypeptide comprising the 4-1BBL amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

10 According to specific embodiments, the article of manufacture comprises the PD1 amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same; and the 4-1BBL amino acid sequence, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

Thus, according to another aspect of the present invention there is provided an article of
15 manufacture comprising a packaging material packaging the isolated polypeptide comprising the PD1 amino acid sequence disclosed herein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same; and the isolated polypeptide comprising the 4-1BBL amino acid sequence disclosed herein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same.

20 According to specific embodiments, the isolated polypeptide comprising the PD1 amino acid sequence disclosed herein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same; and the isolated polypeptide comprising the 4-1BBL amino acid sequence disclosed herein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same are packaged in separate containers.

25 According to specific embodiments, the isolated polypeptide comprising the PD1 amino acid sequence disclosed herein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same; and the isolated polypeptide comprising the 4-1BBL amino acid sequence disclosed herein, a polynucleotide encoding same, a nucleic acid construct encoding same or a host cell expressing same are packaged in a co-formulation.

30 According to specific embodiments, the isolated polypeptide comprising the PD1 amino acid sequence; and/or the isolated polypeptide comprising the 4-1BBL amino acid sequence is attached to or comprises a heterologous therapeutic moiety. The therapeutic moiety may be any molecule, including small molecule chemical compounds and polypeptides.

Non-limiting examples of therapeutic moieties which can be used with specific embodiments of the invention include a cytotoxic moiety, a toxic moiety, a cytokine moiety, an immunomodulatory moiety, a polypeptide, an antibody, a drug, a chemical and/or a radioisotope.

According to some embodiments of the invention, the therapeutic moiety is conjugated by translationally fusing the polynucleotide encoding the polypeptide of some embodiments of the invention with the nucleic acid sequence encoding the therapeutic moiety.

Additionally or alternatively, the therapeutic moiety can be chemically conjugated (coupled) to the polypeptide of some embodiments of the invention, using any conjugation method known to one skilled in the art. For example, a peptide can be conjugated to an agent of interest, using a 3-(2-pyridyldithio) propionic acid Nhydroxysuccinimide ester (also called N-succinimidyl 3-(2-pyridyldithio) propionate) ("SDPD") (Sigma, Cat. No. P-3415; see e.g., Cumber et al. 1985, Methods of Enzymology 112: 207-224), a glutaraldehyde conjugation procedure (see e.g., G.T. Hermanson 1996, "Antibody Modification and Conjugation, in Bioconjugate Techniques, Academic Press, San Diego) or a carbodiimide conjugation procedure [see e.g., J. March, Advanced Organic Chemistry: Reaction's, Mechanism, and Structure, pp. 349-50 & 372-74 (3d ed.), 1985; B. Neises et al. 1978, Angew Chem., Int. Ed. Engl. 17:522; A. Hassner et al. 1978, Tetrahedron Lett. 4475; E.P. Boden et al. 1986, J. Org. Chem. 50:2394 and L.J. Mathias 1979, Synthesis 561].

A therapeutic moiety can be attached, for example, to the polypeptide of some embodiments of the invention using standard chemical synthesis techniques widely practiced in the art [see e.g., [hypertexttransferprotocol://worldwideweb \(dot\) chemistry \(dot\) org/portal/Chemistry](http://worldwideweb(dot)chemistry(dot)org/portal/Chemistry)]], such as using any suitable chemical linkage, direct or indirect, as via a peptide bond (when the functional moiety is a polypeptide), or via covalent bonding to an intervening linker element, such as a linker peptide or other chemical moiety, such as an organic polymer. Chimeric peptides may be linked via bonding at the carboxy (C) or amino (N) termini of the peptides, or via bonding to internal chemical groups such as straight, branched or cyclic side chains, internal carbon or nitrogen atoms, and the like.

As used herein, the terms "protein", "peptide" and "polypeptide", which are interchangeably used herein, encompass native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and peptidomimetics (typically, synthetically synthesized peptides), as well as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body or more capable of penetrating into cells. Such modifications include, but are not limited to N terminus modification, C terminus modification, peptide bond modification, backbone modifications, and residue

modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further details in this respect are provided hereinunder.

5 Peptide bonds (-CO-NH-) within the peptide may be substituted, for example, by N-methylated amide bonds (-N(CH₃)-CO-), ester bonds (-C(=O)-O-), ketomethylene bonds (-CO-CH₂-), sulfinylmethylene bonds (-S(=O)-CH₂-), α -aza bonds (-NH-N(R)-CO-), wherein R is any alkyl (e.g., methyl), amine bonds (-CH₂-NH-), sulfide bonds (-CH₂-S-), ethylene bonds (-CH₂-CH₂-), hydroxyethylene bonds (-CH(OH)-CH₂-), thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), fluorinated olefinic double bonds (-CF=CH-), retro amide bonds (-NH-CO-), peptide derivatives (-N(R)-CH₂-CO-), wherein R is the "normal" side chain, naturally present on the carbon atom.

These modifications can occur at any of the bonds along the peptide chain and even at several (2-3) bonds at the same time.

15 Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted by non-natural aromatic amino acids such as 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic), naphthylalanine, ring-methylated derivatives of Phe, halogenated derivatives of Phe or O-methyl-Tyr.

The peptides of some embodiments of the invention may also include one or more modified amino acids or one or more non-amino acid monomers (e.g. fatty acids, complex carbohydrates etc).

The term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally in vivo, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-amino adipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids.

Tables 1 and 2 below list naturally occurring amino acids (Table 1), and non-conventional or modified amino acids (e.g., synthetic, Table 2) which can be used with some embodiments of the invention.

30 **Table 1**

<i>Amino Acid</i>	<i>Three-Letter Abbreviation</i>	<i>One-letter Symbol</i>
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q

Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any amino acid as above	Xaa	X

Table 2

<i>Non-conventional amino acid</i>	<i>Code</i>	<i>Non-conventional amino acid</i>	<i>Code</i>
ornithine	Orn	hydroxyproline	Hyp
α -aminobutyric acid	Abu	aminonorbornyl-carboxylate	Norb
D-alanine	Dala	aminocyclopropane-carboxylate	Cpro
D-arginine	Darg	N-(3-guanidinopropyl)glycine	Narg
D-asparagine	Dasn	N-(carbamylmethyl)glycine	Nasn
D-aspartic acid	Dasp	N-(carboxymethyl)glycine	Nasp
D-cysteine	Dcys	N-(thiomethyl)glycine	Ncys
D-glutamine	Dgln	N-(2-carbamylethyl)glycine	Ngln
D-glutamic acid	Dglu	N-(2-carboxyethyl)glycine	Nglu
D-histidine	Dhis	N-(imidazolylethyl)glycine	Nhis
D-isoleucine	Dile	N-(1-methylpropyl)glycine	Nile
D-leucine	Dleu	N-(2-methylpropyl)glycine	Nleu
D-lysine	Dlys	N-(4-aminobutyl)glycine	Nlys
D-methionine	Dmet	N-(2-methylthioethyl)glycine	Nmet
D-ornithine	Dorn	N-(3-aminopropyl)glycine	Norn
D-phenylalanine	Dphe	N-benzylglycine	Nphe
D-proline	Dpro	N-(hydroxymethyl)glycine	Nser
D-serine	Dser	N-(1-hydroxyethyl)glycine	Nthr
D-threonine	Dthr	N-(3-indolylethyl)glycine	Nhtrp
D-tryptophan	Dtrp	N-(<i>p</i> -hydroxyphenyl)glycine	Ntyr
D-tyrosine	Dtyr	N-(1-methylethyl)glycine	Nval
D-valine	Dval	N-methylglycine	Nmgly
D-N-methylalanine	Dnmala	L-N-methylalanine	Nmala
D-N-methylarginine	Dnmarg	L-N-methylarginine	Nmarg
D-N-methylasparagine	Dnmasn	L-N-methylasparagine	Nmasn
D-N-methylaspartate	Dnmasp	L-N-methylaspartic acid	Nmasp
D-N-methylcysteine	Dnmcys	L-N-methylcysteine	Nmcys
D-N-methylglutamine	Dnmglu	L-N-methylglutamine	Nmglu

D-N-methylglutamate	Dnmglu	L-N-methylglutamic acid	Nmglu
D-N-methylhistidine	Dnmhis	L-N-methylhistidine	Nmbhis
D-N-methylisoleucine	Dnmile	L-N-methylisoleucine	Nmile
D-N-methylleucine	Dnmleu	L-N-methylleucine	Nmleu
D-N-methyllysine	Dnmlys	L-N-methyllysine	Nmlys
D-N-methylmethionine	Dnmmet	L-N-methylmethionine	Nmmet
D-N-methylornithine	Dnmorn	L-N-methylornithine	Nmorn
D-N-methylphenylalanine	Dnmphe	L-N-methylphenylalanine	Nmphe
D-N-methylproline	Dnmpro	L-N-methylproline	Nmpro
D-N-methylserine	Dnmser	L-N-methylserine	Nmser
D-N-methylthreonine	Dnmthr	L-N-methylthreonine	Nmthr
D-N-methyltryptophan	Dnmtrp	L-N-methyltryptophan	Nmtrp
D-N-methyltyrosine	Dnmtyr	L-N-methyltyrosine	Nmtyr
D-N-methylvaline	Dnmval	L-N-methylvaline	Nmval
L-norleucine	Nle	L-N-methylnorleucine	Nmnle
L-norvaline	Nva	L-N-methylnorvaline	Nmnva
L-ethylglycine	Etg	L-N-methyl-ethylglycine	Nmetg
L-t-butylglycine	Tbug	L-N-methyl-t-butylglycine	Nmtbug
L-homophenylalanine	Hphe	L-N-methyl-homophenylalanine	Nmhphe
α -naphthylalanine	Anap	N-methyl- α -naphthylalanine	Nmanap
penicillamine	Pen	N-methylpenicillamine	Nmpen
γ -aminobutyric acid	Gabu	N-methyl- γ -aminobutyrate	Nmgabu
cyclohexylalanine	Chexa	N-methyl-cyclohexylalanine	Nmchexa
cyclopentylalanine	Cpen	N-methyl-cyclopentylalanine	Nmcpen
α -amino- α -methylbutyrate	Aabu	N-methyl- α -amino- α -methylbutyrate	Nmaabu
α -aminoisobutyric acid	Aib	N-methyl- α -aminoisobutyrate	Nmaib
D- α -methylarginine	Dmarg	L- α -methylarginine	Marg
D- α -methylasparagine	Dmasn	L- α -methylasparagine	Masn
D- α -methylaspartate	Dmasp	L- α -methylaspartate	Masp
D- α -methylcysteine	Dmcys	L- α -methylcysteine	Mcys
D- α -methylglutamine	Dmgln	L- α -methylglutamine	Mgln
D- α -methyl glutamic acid	Dmglu	L- α -methylglutamate	Mglu
D- α -methylhistidine	Dmhis	L- α -methylhistidine	Mhis
D- α -methylisoleucine	Dmile	L- α -methylisoleucine	Mile
D- α -methylleucine	Dmleu	L- α -methylleucine	Mleu
D- α -methyllysine	Dmlys	L- α -methyllysine	Mlys
D- α -methylmethionine	Dmmet	L- α -methylmethionine	Mmet
D- α -methylornithine	Dmorn	L- α -methylornithine	Morn
D- α -methylphenylalanine	Dmphe	L- α -methylphenylalanine	Mphe
D- α -methylproline	Dmpro	L- α -methylproline	Mpro
D- α -methylserine	Dmser	L- α -methylserine	Mser
D- α -methylthreonine	Dmthr	L- α -methylthreonine	Mthr
D- α -methyltryptophan	Dmtrp	L- α -methyltryptophan	Mtrp
D- α -methyltyrosine	Dmtyr	L- α -methyltyrosine	Mtyr
D- α -methylvaline	Dmval	L- α -methylvaline	Mval

N-cyclobutylglycine	Ncbut	L- α -methylnorvaline	Mnva
N-cycloheptylglycine	Nchep	L- α -methylethylglycine	Metg
N-cyclohexylglycine	Nchex	L- α -methyl- <i>t</i> -butylglycine	Mtbug
N-cyclodecylglycine	Ncdec	L- α -methyl-homophenylalanine	Mhphe
N-cyclododecylglycine	Ncdod	α -methyl- α -naphthylalanine	Manap
N-cyclooctylglycine	Ncoct	α -methylpenicillamine	Mpen
N-cyclopropylglycine	Ncpro	α -methyl- γ -aminobutyrate	Mgab
N-cycloundecylglycine	Ncund	α -methyl-cyclohexylalanine	Mchexa
N-(2-aminoethyl)glycine	Naeg	α -methyl-cyclopentylalanine	Mcpen
N-(2,2-diphenylethyl)glycine	Nbhm	N-(N-(2,2-diphenylethyl) carbamylmethyl-glycine	Nnbhm
N-(3,3-diphenylpropyl)glycine	Nbhe	N-(N-(3,3-diphenylpropyl) carbamylmethyl-glycine	Nnbhe
1-carboxy-1-(2,2-diphenylethylamino)cyclopropane	Nmbc	1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid	Tic
phosphoserine	pSer	phosphothreonine	pThr
phosphotyrosine	pTyr	O-methyl-tyrosine	
2-aminoadipic acid		hydroxylysine	

The peptides of some embodiments of the invention are preferably utilized in a linear form, although it will be appreciated that in cases where cyclicization does not severely interfere with peptide characteristics, cyclic forms of the peptide can also be utilized.

5 Since the present peptides are preferably utilized in therapeutics which require the peptides to be in soluble form, the peptides of some embodiments of the invention preferably include one or more non-natural or natural polar amino acids, including but not limited to serine and threonine which are capable of increasing peptide solubility due to their hydroxyl-containing side chain.

10 The amino acids of the peptides of the present invention may be substituted either conservatively or non-conservatively.

The term "conservative substitution" as used herein, refers to the replacement of an amino acid present in the native sequence in the peptide with a naturally or non-naturally occurring amino acid or a peptidomimetic having similar steric properties. Where the side-chain of the native amino acid to be replaced is either polar or hydrophobic, the conservative substitution should be with a naturally occurring amino acid, a non-naturally occurring amino acid or with a peptidomimetic moiety which is also polar or hydrophobic (in addition to having the same steric properties as the side-chain of the replaced amino acid).

As naturally occurring amino acids are typically grouped according to their properties, conservative substitutions by naturally occurring amino acids can be easily determined bearing in

mind the fact that in accordance with the invention replacement of charged amino acids by sterically similar non-charged amino acids are considered as conservative substitutions.

For producing conservative substitutions by non-naturally occurring amino acids it is also possible to use amino acid analogs (synthetic amino acids) well known in the art. A peptidomimetic of the naturally occurring amino acid is well documented in the literature known to the skilled practitioner.

When affecting conservative substitutions, the substituting amino acid should have the same or a similar functional group in the side chain as the original amino acid.

Conservative substitution tables providing functionally similar amino acids are well known in the art. Guidance concerning which amino acid changes are likely to be phenotypically silent can also be found in Bowie et al., 1990, Science 247: 1306-1310. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles. Typical conservative substitutions include but are not limited to: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)). Amino acids can be substituted based upon properties associated with side chains, for example, amino acids with polar side chains may be substituted, for example, Serine (S) and Threonine (T); amino acids based on the electrical charge of a side chain, for example, Arginine (R) and Histidine (H); and amino acids that have hydrophobic side chains, for example, Valine (V) and Leucine (L). As indicated, changes are typically of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein.

The phrase "non-conservative substitutions" as used herein refers to replacement of the amino acid as present in the parent sequence by another naturally or non-naturally occurring amino acid, having different electrochemical and/or steric properties. Thus, the side chain of the substituting amino acid can be significantly larger (or smaller) than the side chain of the native amino acid being substituted and/or can have functional groups with significantly different electronic properties than the amino acid being substituted. Examples of non-conservative substitutions of this type include the substitution of phenylalanine or cyclohexylmethyl glycine for alanine, isoleucine for glycine, or $-NH-CH[(-CH_2)_5-COOH]-CO-$ for aspartic acid. Those non-conservative substitutions which fall under the scope of the present invention are those which still constitute a peptide having anti-bacterial properties.

The N and C termini of the peptides of the present invention may be protected by function groups. Suitable functional groups are described in Green and Wuts, "Protecting Groups in Organic

Synthesis", John Wiley and Sons, Chapters 5 and 7, 1991, the teachings of which are incorporated herein by reference. Preferred protecting groups are those that facilitate transport of the compound attached thereto into a cell, for example, by reducing the hydrophilicity and increasing the lipophilicity of the compounds.

5 According to specific embodiments, one or more of the amino acids may be modified by the addition of a functional group, for example (conceptually views as "chemically modified"). For example, the side amino acid residues appearing in the native sequence may optionally be modified, although as described below alternatively other parts of the protein may optionally be modified, in addition to or in place of the side amino acid residues. The modification may
10 optionally be performed during synthesis of the molecule if a chemical synthetic process is followed, for example by adding a chemically modified amino acid. However, chemical modification of an amino acid when it is already present in the molecule ("in situ" modification) is also possible. Modifications to the peptide or protein can be introduced by gene synthesis, site-directed (e.g., PCR based) or random mutagenesis (e.g., EMS) by exonuclease deletion, by
15 chemical modification, or by fusion of polynucleotide sequences encoding a heterologous domain or binding protein, for example.

As used herein the term "chemical modification", when referring to a peptide, refers to a peptide where at least one of its amino acid residues is modified either by natural processes, such as processing or other post-translational modifications, or by chemical modification techniques
20 which are well known in the art. Non-limiting exemplary types of modification include carboxymethylation, acetylation, acylation, phosphorylation, glycosylation, amidation, ADP-ribosylation, fatty acylation, addition of farnesyl group, an isofarnesyl group, a carbohydrate group, a fatty acid group, a linker for conjugation, functionalization, GPI anchor formation, covalent attachment of a lipid or lipid derivative, methylation, myristylation, pegylation, prenylation,
25 phosphorylation, ubiquitination, or any similar process and known protecting/blocking groups. Ether bonds can optionally be used to join the serine or threonine hydroxyl to the hydroxyl of a sugar. Amide bonds can optionally be used to join the glutamate or aspartate carboxyl groups to an amino group on a sugar (Garg and Jeanloz, *Advances in Carbohydrate Chemistry and Biochemistry*, Vol. 43, Academic Press (1985); Kunz, *Ang. Chem. Int. Ed. English* 26:294-308 (1987)). Acetal
30 and ketal bonds can also optionally be formed between amino acids and carbohydrates. Fatty acid acyl derivatives can optionally be made, for example, by acylation of a free amino group (e.g., lysine) (Toth et al., *Peptides: Chemistry, Structure and Biology*, Rivier and Marshal, eds., ESCOM Publ., Leiden, 1078-1079 (1990)).

According to specific embodiments, the modifications include the addition of a cycloalkane moiety to the peptide, as described in PCT Application No. WO 2006/050262, hereby incorporated by reference as if fully set forth herein. These moieties are designed for use with biomolecules and may optionally be used to impart various properties to proteins.

5 Furthermore, optionally any point on the peptide may be modified. For example, pegylation of a glycosylation moiety on a protein may optionally be performed, as described in PCT Application No. WO 2006/050247, hereby incorporated by reference as if fully set forth herein. One or more polyethylene glycol (PEG) groups may optionally be added to O-linked and/or N-linked glycosylation. The PEG group may optionally be branched or linear. Optionally any type of
10 water-soluble polymer may be attached to a glycosylation site on a protein through a glycosyl linker.

By "PEGylated protein" is meant a protein, or a fragment thereof having biological activity, having a polyethylene glycol (PEG) moiety covalently bound to an amino acid residue of the protein.

15 By "polyethylene glycol" or "PEG" is meant a polyalkylene glycol compound or a derivative thereof, with or without coupling agents or derivatization with coupling or activating moieties (e.g., with thiol, triflate, tresylate, azirdine, oxirane, or preferably with a maleimide moiety). Compounds such as maleimido monomethoxy PEG are exemplary or activated PEG compounds of the invention. Other polyalkylene glycol compounds, such as polypropylene glycol, may be used in the
20 present invention. Other appropriate polyalkylene glycol compounds include, but are not limited to, charged or neutral polymers of the following types: dextran, colominic acids or other carbohydrate-based polymers, polymers of amino acids, and biotin derivatives.

According to specific embodiments, the peptide is modified to have an altered glycosylation pattern (i.e., altered from the original or native glycosylation pattern). As used herein, "altered"
25 means having one or more carbohydrate moieties deleted, and/or having at least one glycosylation site added to the original protein.

Glycosylation of proteins is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences, asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except
30 proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to a peptide is conveniently accomplished by altering the amino acid sequence of the peptide such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues in the sequence of the original peptide (for O-linked glycosylation sites). The peptide's amino acid sequence may also be altered by introducing changes at the DNA level.

Another means of increasing the number of carbohydrate moieties on peptides is by chemical or enzymatic coupling of glycosides to the amino acid residues of the peptide. Depending on the coupling mode used, the sugars may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described e.g. in WO 87/05330, and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, 22: 259-306 (1981).

Removal of any carbohydrate moieties present on a peptide may be accomplished chemically, enzymatically or by introducing changes at the DNA level. Chemical deglycosylation requires exposure of the peptide to trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), leaving the amino acid sequence intact.

Chemical deglycosylation is described by Hakimuddin et al., *Arch. Biochem. Biophys.*, 259: 52 (1987); and Edge et al., *Anal. Biochem.*, 118: 131 (1981). Enzymatic cleavage of carbohydrate moieties on peptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., *Meth. Enzymol.*, 138: 350 (1987).

According to specific embodiments, the peptide comprises a detectable tag. As used herein, in one embodiment the term "detectable tag" refers to any moiety that can be detected by a skilled practitioner using art known techniques. Detectable tags may be peptide sequences. Optionally the detectable tag may be removable by chemical agents or by enzymatic means, such as proteolysis. Detectable tags of some embodiments of the present invention can be used for purification of the peptide. For example the term "detectable tag" includes chitin binding protein (CBP)-tag, maltose binding protein (MBP)-tag, glutathione-S-transferase (GST)-tag, poly(His)-tag, FLAG tag, Epitope tags, such as, V5-tag, c-myc-tag, and HA-tag, and fluorescence tags such as green fluorescent protein (GFP), red fluorescent protein (RFP), yellow fluorescent protein (YFP), blue fluorescent protein (BFP), and cyan fluorescent protein (CFP); as well as derivatives of these tags, or any tag known in the art. The term "detectable tag" also includes the term "detectable marker".

According to specific embodiment, the peptide comprises a detectable tag attached to its N-

terminal (e.g. poly(His)-tag).

According to specific embodiment, the peptide comprises a detectable tag attached to its C-terminal (e.g. poly(His)-tag).

According to specific embodiments, the N-terminal of the peptide does not comprise a detectable tag (e.g. poly(His)-tag).

According to specific embodiments, the C-terminal of the peptide does not comprise a detectable tag (e.g. poly(His)-tag).

According to specific embodiments the peptide is fused to a cleavable moiety. Thus, for example, to facilitate recovery, the expressed coding sequence can be engineered to encode the peptide of some embodiments of the present invention and fused cleavable moiety. In one embodiment, the peptide is designed such that it is readily isolated by affinity chromatography; e.g., by immobilization on a column specific for the cleavable moiety. In one embodiment, a cleavage site is engineered between the peptide and the cleavable moiety and the peptide can be released from the chromatographic column by treatment with an appropriate enzyme or agent that specifically cleaves the fusion protein at this site [e.g., see Booth *et al.*, Immunol. Lett. 19:65-70 (1988); and Gardella *et al.*, J. Biol. Chem. 265:15854-15859 (1990)]. According to specific embodiments, the peptide is an isolated peptide.

The peptides of some embodiments of the invention may be synthesized and purified by any techniques that are known to those skilled in the art of peptide synthesis, such as, but not limited to, solid phase and recombinant techniques.

For solid phase peptide synthesis, a summary of the many techniques may be found in J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, W. H. Freeman Co. (San Francisco), 1963 and J. Meienhofer, Hormonal Proteins and Peptides, vol. 2, p. 46, Academic Press (New York), 1973. For classical solution synthesis see G. Schroder and K. Lupke, The Peptides, vol. 1, Academic Press (New York), 1965.

In general, these methods comprise the sequential addition of one or more amino acids or suitably protected amino acids to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid is protected by a suitable protecting group. The protected or derivatized amino acid can then either be attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence having the complimentary (amino or carboxyl) group suitably protected, under conditions suitable for forming the amide linkage. The protecting group is then removed from this newly added amino acid residue and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining protecting groups (and any solid support) are removed sequentially or concurrently, to afford the final peptide compound. By simple modification of this

general procedure, it is possible to add more than one amino acid at a time to a growing chain, for example, by coupling (under conditions which do not racemize chiral centers) a protected tripeptide with a properly protected dipeptide to form, after deprotection, a pentapeptide and so forth. Further description of peptide synthesis is disclosed in U.S. Pat. No. 6,472,505.

5 A preferred method of preparing the peptide compounds of some embodiments of the invention involves solid phase peptide synthesis.

Large scale peptide synthesis is described by Andersson Biopolymers 2000;55(3):227-50.

According to specific embodiments, the peptide is synthesized using *in vitro* expression systems. Such *in vitro* synthesis methods are well known in the art and the components of the
10 system are commercially available.

According to specific embodiments, the peptide is produced by recombinant DNA technology. A "recombinant" peptide, or protein refers to a peptide, or protein produced by recombinant DNA techniques; i.e., produced from cells transformed by an exogenous DNA construct encoding the desired peptide or protein.

15 Thus, according to another aspect of the present invention, there is provided an isolated polynucleotide comprising a nucleic acid sequence encoding any of the above described polypeptides and fusion proteins.

According to specific embodiments, the polynucleotide is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the nucleic acid sequence as set forth in SEQ
20 ID NO: 57 – 72, 98, 102, 104, 106, 108, 112, 114, 134, 136, 138, 140, 142, 144 and 148, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the nucleic acid sequence as set forth in SEQ
ID NO: 134, 136, 138, 140, 142, 144 and 148, each possibility represents a separate embodiment
25 of the present invention.

According to specific embodiments, the polynucleotide is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the nucleic acid sequence as set forth in SEQ
ID NO: 57 – 72, 98, 102, 104, 106, 108 or 112, each possibility represents a separate embodiment
of the present invention.

30 According to specific embodiments, the polynucleotide is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the nucleic acid sequence as set forth in SEQ
ID NO: 98, 102, 104, 106, 108 or 112, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the nucleic acid sequence as set forth in SEQ ID NO: 98, 102, 104, 106 or 108, each possibility represents a separate embodiment of the present invention.

5 According to specific embodiments, the polynucleotide is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the nucleic acid sequence as set forth in SEQ ID No. 57-72, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the nucleic acid sequence as set forth in SEQ
10 ID NO: 146.

According to specific embodiments, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72, 98, 102, 104, 106, 108, 112, 114, 134, 136, 138, 140, 142, 144 and 148, each possibility represents a separate embodiment of the present invention.

15 According to specific embodiments, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 134, 136, 138, 140, 142, 144 and 148, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72, 98, 102, 104, 106, 108 or 112, each
20 possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 98, 102, 104, 106, 108 or 112, each possibility represents a separate embodiment of the present invention.

According to specific embodiments the polynucleotide comprises a nucleic acid sequence
25 selected from the group consisting of SEQ ID NO: 98, 102, 104, 106 or 108, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72, each possibility represents a separate embodiment of the present invention.

30 According to specific embodiments, the polynucleotide comprises a nucleic acid sequence as set forth in SEQ ID NO: 146.

According to specific embodiments, the polynucleotide consists of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72, 98, 102, 104, 106, 108, 112, 114, 134,

136, 138, 140, 142, 144 and 148, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide consists of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 134, 136, 138, 140, 142, 144 and 148, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide consists of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72, 98, 102, 104, 106, 108 or 112, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide consists of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 98, 102, 104, 106, 108 or 112, each possibility represents a separate embodiment of the present invention.

According to specific embodiments the polynucleotide consists of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 98, 102, 104, 106 or 108, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide consists of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide is least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the nucleic sequence as set forth in SEQ ID No. 70-72.

According to specific embodiments, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 70-72.

According to specific embodiments, the polynucleotide consists of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 70-72.

According to specific embodiments, the polynucleotide consists of SEQ ID NO: 146.

According to another aspect of the present invention, there is provided an isolated polynucleotide comprising a nucleic acid sequence encoding any of the above described PD1 amino acid sequence being 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or being 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and not comprising an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and optionally comprising a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO:2;

120

or any of the above described 4-1BBL amino acid sequence being 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, being 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and not comprising an amino acid segment G198-E205 corresponding to SEQ ID NO: 3, or being 170 – 182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and not comprising an amino acid segment A1-E23 corresponding to SEQ ID NO: 3; and optionally comprising three repeats of the 4-1BBL amino acid sequence.

According to another aspect of the present invention, there is provided an isolated polynucleotide comprising a nucleic acid sequence encoding any of the above described PD1 amino acid sequence being 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and optionally comprising a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; or any of the above described 4-1BBL amino acid sequence being 185 - 202 amino acids in length and having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25.

According to additional or an alternative aspect of the present invention, there is provided an isolated polynucleotide comprising a nucleic acid sequence encoding any of the above described PD1 amino acid sequence being 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21 and not comprising any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; or any of the above described 4-1BBL amino acid sequence being 185 - 202 amino acids in length and having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25.

According to specific embodiments, the polynucleotide is at least 70 %, 75 %, 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 88 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % or 99 % homologous to the nucleic acid sequence as set forth in SEQ ID NO: 32, 33, 34, 35, 74, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 118 or 120, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide is at least 70 %, 75 %, 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 88 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % or 99 % homologous to the nucleic sequence as set forth in SEQ ID NO: 32, 33, 34, 35,

74, 76, 80, 82, 84, 86, 88, 90, 92, 94 or 96, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide is at least about 95%, 96%, 97%, 98% or 99% homologous to the nucleic acid sequence as set forth in SEQ ID NO: 32, 33, 34, 35, 74, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 118 or 120, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide is at least about 95%, 96%, 97%, 98% or 99% homologous to the nucleic sequence as set forth in SEQ ID NO: 32, 33, 34, 35, 74, 76, 80, 82, 84, 86, 88, 90, 92, 94 or 96.

According to specific embodiments, the polynucleotide comprises SEQ ID NO: 32, 33, 34, 35, 74, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 118 or 120. According to specific embodiments, the polynucleotide comprises SEQ ID NO: 32, 33, 34, 35, 74, 76, 80, 82, 84, 86, 88, 90, 92, 94 or 96.

According to specific embodiments, the polynucleotide consists of SEQ ID NO: 32, 33, 34, 35, 74, 76, 80, 82, 84, 86, 88, 90, 92, 94, 96, 116, 118 or 120.

According to specific embodiments, the polynucleotide consists of SEQ ID NO: 32, 33, 34, 35, 74, 76, 80, 82, 84, 86, 88, 90, 92, 94 or 96.

According to specific embodiments, the polynucleotide is least 70 %, 75 %, 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 88 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % or 99 % homologous to the nucleic sequence as set forth in SEQ ID NO: 74, 76, 80, 82, 84, 86, 88, 90, 92, 94 or 96, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide is least about 95%, 96%, 97%, 98% or 99% homologous to the nucleic sequence as set forth in SEQ ID NO: 74, 76, 80, 82, 84, 86, 88, 90, 92, 94 or 96.

According to specific embodiments, the polynucleotide comprises SEQ ID NO: 74, 76, 80, 82, 84, 86, 88, 90, 92, 94 or 96.

According to specific embodiments, the polynucleotide consists of SEQ ID NO: 74, 76, 80, 82, 84, 86, 88, 90, 92, 94 or 96.

According to specific embodiments, the polynucleotide is least 70 %, 75 %, 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 88 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % or 99 % homologous to the nucleic sequence as set forth in SEQ ID NO: 32, 33, 34 or 35, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide is least about 95%, 96%, 97%, 98% or 99% homologous to the nucleic sequence as set forth in SEQ ID NO: 32, 33, 34 or 35.

According to specific embodiments, the polynucleotide comprises SEQ ID NO: 32, 33, 34 or 35.

5 According to specific embodiments, the polynucleotide consists of SEQ ID NO: 32, 33, 34 or 35.

According to specific embodiments, the polynucleotide is at least 70 %, 75 %, 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 88 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % or 99 % homologous to the nucleic acid sequence as set forth in SEQ ID NO: 39, 40,
10 41, 42, 126, 128, 130 or 132 each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide is at least 70 %, 75 %, 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 88 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % or 99 % homologous to the nucleic sequence as set forth in SEQ ID NO: 39, 40, 41 or
15 42, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polynucleotide is at least about 95%, 96%, 97%, 98% or 99% homologous to the nucleic sequence as set forth in SEQ ID NO: 39, 40, 41, 42, 126, 128, 130 or 132.

According to specific embodiments, the polynucleotide is at least about 95%, 96%, 97%, 98%
20 or 99% homologous to the nucleic sequence as set forth in SEQ ID NO: 39, 40, 41 or 42.

According to specific embodiments, the polynucleotide comprises SEQ ID NO: 39, 40, 41, 42, 126, 128, 130 or 132.

According to specific embodiments, the polynucleotide comprises SEQ ID NO: 39, 40, 41 or 42.

25 According to specific embodiments, the polynucleotide consists of SEQ ID NO: 39, 40, 41, 42, 126, 128, 130 or 132.

According to specific embodiments, the polynucleotide consists of SEQ ID NO: 39, 40, 41 or 42.

As used herein the term “polynucleotide” refers to a single or double stranded nucleic acid
30 sequence which is isolated and provided in the form of an RNA sequence, a complementary polynucleotide sequence (cDNA), a genomic polynucleotide sequence and/or a composite polynucleotide sequences (e.g., a combination of the above).

According to specific embodiments, any of the polynucleotides and nucleic acid sequences disclosed herein may comprise conservative nucleic acid substitutions. Conservatively modified

polynucleotides refer to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical or associated (e.g., naturally contiguous) sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode most proteins. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to another of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations", which are one species of conservatively modified polynucleotides. According to specific embodiments, any polynucleotide and nucleic acid sequence described herein which encodes a polypeptide also describes silent variations of the nucleic acid. One of skill will recognize that in certain contexts each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, silent variations of a polynucleotide which encodes a polypeptide is implicit in a described sequence with respect to the expression product.

To express an exogenous polypeptide in mammalian cells, a polynucleotide sequence encoding the polypeptide is preferably ligated into a nucleic acid construct suitable for mammalian cell expression. Such a nucleic acid construct includes a promoter sequence for directing transcription of the polynucleotide sequence in the cell in a constitutive or inducible manner.

Hence, according to specific embodiments, there is provided nucleic acid construct comprising the polynucleotide and a regulatory element for directing expression of said polynucleotide in a host cell.

According to specific embodiments, the regulatory element is a heterologous regulatory element.

The nucleic acid construct (also referred to herein as an "expression vector") of some embodiments of the invention includes additional sequences which render this vector suitable for replication and integration in prokaryotes, eukaryotes, or preferably both (e.g., shuttle vectors). In addition, a typical cloning vector may also contain a transcription and translation initiation sequence, transcription and translation terminator and a polyadenylation signal. By way of example, such constructs will typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3' LTR or a portion thereof.

The nucleic acid construct of some embodiments of the invention typically includes a signal sequence for secretion of the peptide from a host cell in which it is placed. Preferably the signal

sequence for this purpose is a mammalian signal sequence or the signal sequence of the polypeptide variants of some embodiments of the invention.

Eukaryotic promoters typically contain two types of recognition sequences, the TATA box and upstream promoter elements. The TATA box, located 25-30 base pairs upstream of the transcription initiation site, is thought to be involved in directing RNA polymerase to begin RNA synthesis. The other upstream promoter elements determine the rate at which transcription is initiated.

Preferably, the promoter utilized by the nucleic acid construct of some embodiments of the invention is active in the specific cell population transformed. Examples of cell type-specific and/or tissue-specific promoters include promoters such as albumin that is liver specific [Pinkert et al., (1987) *Genes Dev.* 1:268-277], lymphoid specific promoters [Calame et al., (1988) *Adv. Immunol.* 43:235-275]; in particular promoters of T-cell receptors [Winoto et al., (1989) *EMBO J.* 8:729-733] and immunoglobulins; [Banerji et al. (1983) *Cell* 33:729-740], neuron-specific promoters such as the neurofilament promoter [Byrne et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477], pancreas-specific promoters [Edlunch et al. (1985) *Science* 230:912-916] or mammary gland-specific promoters such as the milk whey promoter (U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166).

Enhancer elements can stimulate transcription up to 1,000 fold from linked homologous or heterologous promoters. Enhancers are active when placed downstream or upstream from the transcription initiation site. Many enhancer elements derived from viruses have a broad host range and are active in a variety of tissues. For example, the SV40 early gene enhancer is suitable for many cell types. Other enhancer/promoter combinations that are suitable for some embodiments of the invention include those derived from polyoma virus, human or murine cytomegalovirus (CMV), the long term repeat from various retroviruses such as murine leukemia virus, murine or Rous sarcoma virus and HIV. See, *Enhancers and Eukaryotic Expression*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 1983, which is incorporated herein by reference.

In the construction of the expression vector, the promoter is preferably positioned approximately the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

Polyadenylation sequences can also be added to the expression vector in order to increase the efficiency of PD1-4-1BBL, PD1 or 4-1BBL mRNA translation. Two distinct sequence elements are required for accurate and efficient polyadenylation: GU or U rich sequences located downstream from the polyadenylation site and a highly conserved sequence of six nucleotides,

AAUAAA, located 11-30 nucleotides upstream. Termination and polyadenylation signals that are suitable for some embodiments of the invention include those derived from SV40.

In addition to the elements already described, the expression vector of some embodiments of the invention may typically contain other specialized elements intended to increase the level of expression of cloned nucleic acids or to facilitate the identification of cells that carry the recombinant DNA. For example, a number of animal viruses contain DNA sequences that promote the extra chromosomal replication of the viral genome in permissive cell types. Plasmids bearing these viral replicons are replicated episomally as long as the appropriate factors are provided by genes either carried on the plasmid or with the genome of the host cell.

The vector may or may not include a eukaryotic replicon. If a eukaryotic replicon is present, then the vector is amplifiable in eukaryotic cells using the appropriate selectable marker. If the vector does not comprise a eukaryotic replicon, no episomal amplification is possible. Instead, the recombinant DNA integrates into the genome of the engineered cell, where the promoter directs expression of the desired nucleic acid.

The expression vector of some embodiments of the invention can further include additional polynucleotide sequences that allow, for example, the translation of several proteins from a single mRNA such as an internal ribosome entry site (IRES) and sequences for genomic integration of the promoter-chimeric polypeptide.

It will be appreciated that the individual elements comprised in the expression vector can be arranged in a variety of configurations. For example, enhancer elements, promoters and the like, and even the polynucleotide sequence(s) encoding a PD1-4-1BBL, a polypeptide comprising a PD1 amino acid sequence or a polypeptide comprising a 4-1BBL amino acid sequence can be arranged in a "head-to-tail" configuration, may be present as an inverted complement, or in a complementary configuration, as an anti-parallel strand. While such variety of configuration is more likely to occur with non-coding elements of the expression vector, alternative configurations of the coding sequence within the expression vector are also envisioned.

Examples for mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pGL3, pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, pNMT81, which are available from Invitrogen, pCI which is available from Promega, pMbac, pPbac, pBK-RSV and pBK-CMV which are available from Stratagene, pTRES which is available from Clontech, and their derivatives.

Expression vectors containing regulatory elements from eukaryotic viruses such as retroviruses can be also used. SV40 vectors include pSVT7 and pMT2. Vectors derived from bovine papilloma virus include pBV-1MTHA, and vectors derived from Epstein Bar virus include

pHEBO, and p2O5. Other exemplary vectors include pMSG, pAV009/A+, pMTO10/A+, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV-40 early promoter, SV-40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. Thus, the type of vector used by some embodiments of the invention will depend on the cell type transformed. The ability to select suitable vectors according to the cell type transformed is well within the capabilities of the ordinary skilled artisan and as such no general description of selection consideration is provided herein. For example, bone marrow cells can be targeted using the human T cell leukemia virus type I (HTLV-I) and kidney cells may be targeted using the heterologous promoter present in the baculovirus *Autographa californica* nucleopolyhedrovirus (AcMNPV) as described in Liang CY et al., 2004 (Arch Virol. 149: 51-60).

Recombinant viral vectors are useful for in vivo expression of PD1-4-1BBL, PD1 or 4-1BBL since they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

Various methods can be used to introduce the expression vector of some embodiments of the invention into cells. Such methods are generally described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., *Somatic Gene Therapy*, CRC Press, Ann Arbor, Mich. (1995), Vega et al., *Gene Targeting*, CRC Press, Ann Arbor Mich. (1995), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston Mass. (1988) and Gilboa et al. [Biotechniques 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection, electroporation and infection

with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

Introduction of nucleic acids by viral infection offers several advantages over other methods such as lipofection and electroporation, since higher transfection efficiency can be obtained due to the infectious nature of viruses.

Currently preferred in vivo nucleic acid transfer techniques include transfection with viral or non-viral constructs, such as adenovirus, lentivirus, Herpes simplex I virus, or adeno-associated virus (AAV) and lipid-based systems. Useful lipids for lipid-mediated transfer of the gene are, for example, DOTMA, DOPE, and DC-Chol [Tonkinson et al., Cancer Investigation, 14(1): 54-65 (1996)]. The most preferred constructs for use in gene therapy are viruses, most preferably adenoviruses, AAV, lentiviruses, or retroviruses. A viral construct such as a retroviral construct includes at least one transcriptional promoter/enhancer or locus-defining element(s), or other elements that control gene expression by other means such as alternate splicing, nuclear RNA export, or post-translational modification of messenger. Such vector constructs also include a packaging signal, long terminal repeats (LTRs) or portions thereof, and positive and negative strand primer binding sites appropriate to the virus used, unless it is already present in the viral construct. In addition, such a construct typically includes a signal sequence for secretion of the peptide from a host cell in which it is placed. Preferably the signal sequence for this purpose is a mammalian signal sequence or the signal sequence of the polypeptide variants of some embodiments of the invention. Optionally, the construct may also include a signal that directs polyadenylation, as well as one or more restriction sites and a translation termination sequence. By way of example, such constructs will typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3' LTR or a portion thereof. Other vectors can be used that are non-viral, such as cationic lipids, polylysine, and dendrimers.

As mentioned, other than containing the necessary elements for the transcription and translation of the inserted coding sequence, the expression construct of some embodiments of the invention can also include sequences engineered to enhance stability, production, purification, yield or toxicity of the expressed peptide. For example, the expression of a fusion protein or a cleavable fusion protein comprising the PD1-4-1BBL protein or the polypeptide of some embodiments of the invention and a heterologous protein can be engineered. Such a fusion protein can be designed so that the fusion protein can be readily isolated by affinity chromatography; e.g., by immobilization on a column specific for the heterologous protein. Where a cleavage site is engineered between the PD1-4-1BBL protein or the polypeptide of some embodiments of the present invention and the heterologous protein, the PD1-4-1BBL protein or the polypeptide can be

released from the chromatographic column by treatment with an appropriate enzyme or agent that disrupts the cleavage site [e.g., see Booth et al. (1988) *Immunol. Lett.* 19:65-70; and Gardella et al., (1990) *J. Biol. Chem.* 265:15854-15859].

The present invention also contemplates cells comprising the composition described herein.

5 Thus, according to specific embodiments, there is provided a host cell comprising the PD1-4-1BBL fusion protein, the polypeptide comprising the PD1 amino acid sequence and/or the polypeptide comprising the 4-1BBL amino acid sequence disclosed herein, the polynucleotide encoding same or the nucleic acid construct encoding same.

As mentioned hereinabove, a variety of prokaryotic or eukaryotic cells can be used as host-
10 expression systems to express the polypeptides of some embodiments of the invention. These include, but are not limited to, microorganisms, such as bacteria transformed with a recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vector containing the coding sequence; yeast transformed with recombinant yeast expression vectors containing the coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower
15 mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors, such as Ti plasmid, containing the coding sequence. Mammalian expression systems can also be used to express the polypeptides of some embodiments of the invention.

Examples of bacterial constructs include the pET series of *E. coli* expression vectors [Studier et al. (1990) *Methods in Enzymol.* 185:60-89].

20 Examples of eukaryotic cells which may be used along with the teachings of the invention include but are not limited to, mammalian cells, fungal cells, yeast cells, insect cells, algal cells or plant cells.

In yeast, a number of vectors containing constitutive or inducible promoters can be used, as disclosed in U.S. Pat. Application No: 5,932,447. Alternatively, vectors can be used which
25 promote integration of foreign DNA sequences into the yeast chromosome.

In cases where plant expression vectors are used, the expression of the coding sequence can be driven by a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV [Brisson et al. (1984) *Nature* 310:511-514], or the coat protein promoter to TMV [Takamatsu et al. (1987) *EMBO J.* 6:307-311] can be used. Alternatively, plant promoters
30 such as the small subunit of RUBISCO [Coruzzi et al. (1984) *EMBO J.* 3:1671-1680 and Brogli et al., (1984) *Science* 224:838-843] or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B [Gurley et al. (1986) *Mol. Cell. Biol.* 6:559-565] can be used. These constructs can be introduced into plant cells using Ti plasmid, Ri plasmid, plant viral vectors, direct DNA transformation, microinjection, electroporation and other techniques well known to the skilled artisan. See, for

example, Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463.

Other expression systems such as insects and mammalian host cell systems which are well known in the art can also be used by some embodiments of the invention.

5 According to specific embodiments the cell is a mammalian cell.

According to specific embodiment, the cell is a human cell.

According to a specific embodiment, the cell is a cell line.

According to another specific embodiment, the cell is a primary cell.

10 The cell may be derived from a suitable tissue including but not limited to blood, muscle, nerve, brain, heart, lung, liver, pancreas, spleen, thymus, esophagus, stomach, intestine, kidney, testis, ovary, hair, skin, bone, breast, uterus, bladder, spinal cord, or various kinds of body fluids. The cells may be derived from any developmental stage including embryo, fetal and adult stages, as well as developmental origin i.e., ectodermal, mesodermal, and endodermal origin.

Non limiting examples of mammalian cells include monkey kidney CV1 line transformed by
15 SV40 (COS, e.g. COS-7, ATCC CRL 1651); human embryonic kidney line (HEK293 or HEK293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol., 36:59 1977); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 1980); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HeLa, ATCC CCL 2);
20 NIH3T3, Jurkat, canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci., 383:44-68 1982); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2), PER.C6, K562, and Chinese hamster ovary cells (CHO).

25 According to some embodiments of the invention, the mammalian cell is selected from the group consisting of a Chinese Hamster Ovary (CHO), HEK293, PER.C6, HT1080, NS0, Sp2/0, BHK, Namalwa, COS, HeLa and Vero cell.

According to some embodiments of the invention, the host cell comprises a Chinese Hamster Ovary (CHO), PER.C6 or a 293 (e.g. Expi293F) cell.

30 According to another aspect of the present invention, there is provided a method of producing a PD1-4-1BBL fusion protein, a polypeptide comprising a PD1 amino acid sequence or a polypeptide comprising a 4-1BBL amino acid sequence, the method comprising expressing in a host cell the polynucleotide or the nucleic acid construct described herein.

According to specific embodiments, the methods comprising isolating the fusion protein or the polypeptide.

According to specific embodiments, recovery of the recombinant polypeptide is effected following an appropriate time in culture. The phrase "recovering the recombinant polypeptide" refers to collecting the whole fermentation medium containing the polypeptide and need not imply additional steps of separation or purification. Notwithstanding the above, polypeptides of some embodiments of the invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, mix mode chromatography, metal affinity chromatography, Lectins affinity chromatography chromatofocusing and differential solubilization.

According to specific embodiments, following synthesis and purification, the therapeutic efficacy of the peptide can be assayed either in vivo or in vitro. Such methods are known in the art and include for example cell viability, survival of transgenic mice, and expression of activation markers .

The compositions (e.g. the PD1-4-1BBL fusion protein, the polypeptide comprising the PD1 amino acid sequence or the polypeptide comprising the 4-1BBL amino acid sequence disclosed herein, polynucleotide encoding same, nucleic acid construct encoding same and/or cells) of some embodiments of the invention can be administered to an organism *per se*, or in a pharmaceutical composition where it is mixed with suitable carriers or excipients.

Thus, the present invention, in some embodiments, features a pharmaceutical composition comprising a therapeutically effective amount of the composition disclosed herein.

Herein the term "active ingredient" refers to the composition (e.g. PD1-4-1BBL fusion protein, polypeptide comprising a PD1 amino acid sequence, polypeptide comprising a 4-1BBL amino acid sequence, polynucleotide, nucleic acid construct and/or cells described herein) accountable for the biological effect.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the active compound, i.e., a polypeptide, a polynucleotide, a nucleic acid construct and/or cell as described herein, may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see e.g., Berge, S. M., et al. (1977) J. Pharm. Sci. 66: 1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chlorprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A pharmaceutical composition according to at least some embodiments of the present invention also may include a pharmaceutically acceptable anti-oxidants. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like. A pharmaceutical composition according to at least some embodiments of the present invention also may include additives such as detergents and solubilizing agents (e.g., TWEEN 20 (polysorbate-20), TWEEN 80 (polysorbate-80)) and preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol).

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions according to at least some embodiments of the present invention include water, buffered saline of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and

the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate.

Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions according to at least some embodiments of the present invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the

active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.01 per cent to about ninety-nine percent of active ingredient, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent of active ingredient in combination with a pharmaceutically acceptable carrier.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms according to at least some embodiments of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Pharmaceutical compositions of some embodiments of the invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration for therapeutic agents according to at least some embodiments of the present invention include intravascular delivery (e.g. injection or infusion), intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal, oral, enteral, rectal, pulmonary (e.g. inhalation), nasal, topical (including transdermal, buccal and sublingual), intravesical, intravitreal, intraperitoneal, vaginal, brain delivery (e.g. intra-cerebroventricular, intra-cerebral, and convection enhanced diffusion), CNS delivery (e.g. intrathecal, perispinal, and intra-spinal) or parenteral (including subcutaneous, intramuscular, intraperitoneal, intravenous (IV) and intradermal), transdermal (either passively or using iontophoresis or electroporation), transmucosal (e.g., sublingual administration, nasal, vaginal, rectal, or sublingual), administration or administration via an implant, or other parenteral routes of administration, for example by injection or infusion, or other delivery routes and/or forms of administration known in the art. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion or using bioerodible inserts, and can be formulated in dosage forms appropriate for each route of administration. In a specific embodiment, a protein, a therapeutic agent or a pharmaceutical composition according to at least some embodiments of the present invention can be administered intraperitoneally or intravenously.

According to specific embodiments, the compositions disclosed herein are administered in an aqueous solution, by parenteral injection. The formulation may also be in the form of a suspension or emulsion. In general, pharmaceutical compositions for parenteral injection are provided including effective amounts of the compositions described herein, and optionally include pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or

carriers. Such compositions optionally include one or more for the following: diluents, sterile water, buffered saline of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; and additives such as detergents and solubilizing agents (e.g., TWEEN 20 (polysorbate-20), TWEEN 80 (polysorbate-80)), anti-oxidants (e.g., water soluble antioxidants such as ascorbic acid, sodium metabisulfite, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid), and preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol). Examples of non-aqueous solvents or vehicles are ethanol, propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. The formulations may be freeze dried (lyophilized) or vacuum dried and redissolved/resuspended immediately before use. The formulation may be sterilized by, for example, filtration through a bacteria retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions.

Various compositions (e.g., polypeptides) disclosed herein can be applied topically. Topical administration does not work well for most peptide formulations, although it can be effective especially if applied to the lungs, nasal, oral (sublingual, buccal), vaginal, or rectal mucosa.

Compositions of the present invention can be delivered to the lungs while inhaling and traverse across the lung epithelial lining to the blood stream when delivered either as an aerosol or spray dried particles having an aerodynamic diameter of less than about 5 microns. A wide range of mechanical devices designed for pulmonary delivery of therapeutic products can be used, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art. Some specific examples of commercially available devices are the Ultravent nebulizer (Mallinckrodt Inc., St. Louis, Mo.); the Acorn II nebulizer (Marquest Medical Products, Englewood, Colo.); the Ventolin metered dose inhaler (Glaxo Inc., Research Triangle Park, N.C.); and the Spinhaler powder inhaler (Fisons Corp., Bedford, Mass.). Nektar, Alkermes and Mannkind all have inhalable insulin powder preparations approved or in clinical trials where the technology could be applied to the formulations described herein.

Formulations for administration to the mucosa will typically be spray dried drug particles, which may be incorporated into a tablet, gel, capsule, suspension or emulsion. Standard pharmaceutical excipients are available from any formulator. Oral formulations may be in the form of chewing gum, gel strips, tablets or lozenges.

Transdermal formulations may also be prepared. These will typically be ointments, lotions, sprays, or patches, all of which can be prepared using standard technology. Transdermal formulations will require the inclusion of penetration enhancers. Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

According to specific embodiments, the compositions disclosed herein are administered to a subject in a therapeutically effective amount. As used herein the term "effective amount" or "therapeutically effective amount" means a dosage sufficient to treat, inhibit, or alleviate one or more symptoms of the disorder being treated or to otherwise provide a desired pharmacologic and/or physiologic effect. The precise dosage will vary according to a variety of factors such as subject-dependent variables (e.g., age, immune system health, etc.), the disease, and the treatment being effected. For the polypeptide compositions disclosed herein, the polynucleotides and nucleic acids constructs encoding same and the cells described herein, as further studies are conducted, information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients, and the ordinary skilled worker, considering the therapeutic context, age, and general health of the recipient, will be able to ascertain proper dosing. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment desired. For polypeptide compositions, generally dosage levels of 0.0001 to 100 mg/kg of body weight daily are administered to mammals and more usually 0.001 to 20 mg/kg. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration 5 times per week, 4 times per week, 3 times per week, 2 times per week, once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Generally, for intravenous injection or infusion, dosage may be lower. Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be

administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated, each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms according to at least some embodiments of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Optionally the polypeptide formulation may be administered in an amount between 0.0001 to 100 mg/kg weight of the patient/day, preferably between 0.001 to 20.0 mg/kg/day, according to any suitable timing regimen. A therapeutic composition according to at least some embodiments according to at least some embodiments of the present invention can be administered, for example, three times a day, twice a day, once a day, three times weekly, twice weekly or once weekly, once every two weeks or 3, 4, 5, 6, 7 or 8 weeks. Moreover, the composition can be administered over a short or long period of time (e.g., 1 week, 1 month, 1 year, 5 years).

Alternatively, the compositions disclosed herein can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the therapeutic agent in the patient. In general, human antibodies show the longest half-life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The half-life for fusion proteins may vary widely. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

In certain embodiments, the polypeptide, polynucleotide, nucleic acid construct or cells compositions are administered locally, for example by injection directly into a site to be treated. Typically, the injection causes an increased localized concentration of the composition which is greater than that which can be achieved by systemic administration. The polypeptide compositions

can be combined with a matrix as described above to assist in creating an increased localized concentration of the polypeptide compositions by reducing the passive diffusion of the polypeptides out of the site to be treated.

Pharmaceutical compositions of the present invention may be administered with medical devices known in the art. For example, in an optional embodiment, a pharmaceutical composition according to at least some embodiments of the present invention can be administered with a needles hypodermic injection device, such as the devices disclosed in U.S. Pat. Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Pat. No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Pat. No. 4,486,194, which discloses a therapeutic device for administering medicaments through the skin; U.S. Pat. No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Pat. No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Pat. No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Pat. No. 4,475,196, which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

Controlled release polymeric devices can be made for long term release systemically following implantation of a polymeric device (rod, cylinder, film, disk) or injection (microparticles). The matrix can be in the form of microparticles such as microspheres, where peptides are dispersed within a solid polymeric matrix or microcapsules, where the core is of a different material than the polymeric shell, and the peptide is dispersed or suspended in the core, which may be liquid or solid in nature. Unless specifically defined herein, microparticles, microspheres, and microcapsules are used interchangeably. Alternatively, the polymer may be cast as a thin slab or film, ranging from nanometers to four centimeters, a powder produced by grinding or other standard techniques, or even a gel such as a hydrogel.

Either non-biodegradable or biodegradable matrices can be used for delivery of the active agents disclosed herein, although biodegradable matrices are preferred. These may be natural or synthetic polymers, although synthetic polymers are preferred due to the better characterization of degradation and release profiles. The polymer is selected based on the period over which release is desired. In some cases linear release may be most useful, although in others a pulse release or "bulk release" may provide more effective results. The polymer may be in the form of a hydrogel (typically in absorbing up to about 90% by weight of water), and can optionally be crosslinked with multivalent ions or polymers.

The matrices can be formed by solvent evaporation, spray drying, solvent extraction and other methods known to those skilled in the art. Bioerodible microspheres can be prepared using any of the methods developed for making microspheres for drug delivery, for example, as described by Mathiowitz and Langer, *J. Controlled Release*, 5:13-22 (1987); Mathiowitz, et al., *Reactive Polymers*, 6:275-283 (1987); and Mathiowitz, et al., *J. Appl Polymer Sci*, 35:755-774 (1988).

The devices can be formulated for local release to treat the area of implantation or injection - which will typically deliver a dosage that is much less than the dosage for treatment of an entire body - or systemic delivery. These can be implanted or injected subcutaneously, into the muscle, fat, or swallowed.

In certain embodiments, to ensure that the therapeutic compounds according to at least some embodiments of the present invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, e.g., V. V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, e.g., U.S. Pat. No. 5,416,016 to Low et al.); mannosides (Umezawa et al., (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P. G. Bloeman et al. (1995) *FEBS Lett.* 357:140; M. Owais et al. (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe et al. (1995) *Am. J Physiol.* 1233:134); p120 (Schreier et al. (1994) *J. Biol. Chem.* 269:9090); see also K. Keinänen; M. L. Laukkanen (1994) *FEBS Lett.* 346:123; J. J. Killian; I. J. Fidler (1994) *Immunomethods* 4:273.

Compositions of some embodiments of the invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the

container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as is further detailed above.

As used herein the term "about" refers to $\pm 10\%$.

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and

procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

When reference is made to particular sequence listings, such reference is to be understood to also encompass sequences that substantially correspond to its complementary sequence as including minor sequence variations, resulting from, e.g., sequencing errors, cloning errors, or other alterations resulting in base substitution, base deletion or base addition, provided that the frequency of such variations is less than 1 in 50 nucleotides, alternatively, less than 1 in 100 nucleotides, alternatively, less than 1 in 200 nucleotides, alternatively, less than 1 in 500 nucleotides, alternatively, less than 1 in 1000 nucleotides, alternatively, less than 1 in 5,000 nucleotides, alternatively, less than 1 in 10,000 nucleotides.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed.

(1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

SELECTION OF PD1-4-1BBL VARIANTS

A structural analysis of a PD1-4-1BBL fusion protein referred to herein as "DSP105" comprising an N-terminal signal peptide and a his-tag (SEQ ID NO: 26, Figure 1) was effected in order to optimize the following parameters:

- Folding – proper folding to allow binding to targets, minimize potential di-sulfide scrambling
 - Integrity - no exposed proteolytic sites;
 - Multimerization - explore potential multimerization of the two domains.
- Specifically, optimize trimerization of C-terminal domain formation;
- High expression in mammalian expression system; and
 - Low immunogenicity.

Specifically, for the PD1 domain (corresponding to amino acids 21-170 of UniProt ID Q15116 Extracellular domain, SEQ ID NO: 2):

1. A PD1 comprehensive model was generated based on PDB structures: 3RRQ, 5GGR, 5GGS, 5IUS, 5JXE, 4ZQK, 5B8C, 5WT9 and one NMR structure 2M2D. The C-terminal part of PD1 which is missing in the above mentioned PDB structures (starting from GLU150, i.e. EVPTAHPSPSRPAGQFQTLV, SEQ ID NO: 6) includes a Pro-rich segment which is expected to be folded onto itself (according to peptide modelling algorithms).
2. The fusion protein was analyzed for proteolytic sites using the PROSPER server.

For 4-1BBL (corresponding to amino acids 50-254 of UniProt ID P41273 EM domain, SEQ ID NO: 3):

1. A 4-1BBL extracellular (EC) domain model was generated based on PDB structure: 2X29. Since the N-terminal part of the 4-1BBL is missing in this PDB structure and was not resolved in the X-ray, it seems that this segment (ACPWAVSGARASPGSAASPRREGPELSPD, SEQ ID NO: 7) exposes hydrophobic residues to the solvent and attempts to predict its structure indicated an unstructured region. This might lower the stability of the fusion DSP105 and also might interfere with the proper orientation for trimerization via 4-1BBL.
2. The fusion protein was analyzed for proteolytic sites using the PROSPER server.
3. A loop within the 4-1BBL resolved domain was detected which is facing outwards toward the solvent, implying it could be a region which undergoes processing.

Figures 2A-3 and Table 3 below demonstrate the 3D models generated, the domains and segments identified, and the predicted proteolytic sites detected in the analysis of DSP105 fusion protein.

Taken together, the structural analysis indicated the following:

1. Removing a segment of five residues at the N-terminal of PD1 (PGWFL, SEQ ID NO: 8) is recommended since these are mainly hydrophobic by nature, they are not required for recognizing PDL1 and they span outside of the structured domain.
2. Removing a segment of five residues at the C-terminal of PD1 it is also recommended (FQTLV, SEQ ID NO: 9) due to similar apparent behavior as explained in point 1 above (i.e. these are mainly hydrophobic by nature, they are not required for recognizing PDL1 and they span outside of the structured domain).
3. There are 4 predicted cleavage sites found (focusing only on unstructured segments), on the C-terminal of PD1 and on the N-terminal of 4-1BBL.
4. Removing an N-terminal segment of the 4-1BBL domain (ACPWAVSGARASPG, SEQ ID NO: 10 / ACPWAV, SEQ ID NO: 11) is expected to lower flexibility and hydrophobicity of DSP105.
5. In this removed N-terminal segment there is a free Cys residue which might prevent proper folding/rearrangement due to incorrect disulphide bonds.

Table 3: Predicted proteolytic sites in DSP105 PD1-4-1BBL fusion protein (SEQ ID NO: 1)

Merops ID	Protease Name	Position	P4-P4' site	N-fragment (kDa)	C-fragment (kDa)	Cleavage score
M10.003	matrix metalloproteinase-2	144	RPAG QFQT	19.03	24.84	0.99
M10.004	matrix metalloproteinase-9	144	RPAG QFQT	19.03	24.84	0.96
C01.036	cathepsin K	147	GQFQ TLVG	19.44	24.44	1.1
C02.001	calpain-1	151	TLVG ACPW	19.92	23.95	0.94
S01.269	glutamyl peptidase I	174	RLRE GPEL	22.43	21.44	1.04

Based on the structural analysis several PD1 variants were designed having an amino acid sequence of SEQ ID NO: 18, 19, 20, 21, 73, 75, 79, 81, 83, 85, 87, 89, 91, 93, 95, 115, 117 or 119 which can be fused to 4-1BBL or any variant thereof.

In addition, based on the structural analysis several 4-1BBL variants were designed having an amino acid sequence of SEQ ID NO: 22, 23, 24, 25, 123, 125, 127 or 129 which can be fused to PD1 or any variant thereof.

Moreover, to avoid the need of trimerization of the fusion protein to induce activity of the 4-1BBL moiety, 4-1BBL variants comprising 3 repeats of 4-1BBL amino acid sequence were designed, which can be fused to PD1 or any variant thereof. An exemplary sequence of such a 4-1BBL variant comprises three repeats of SEQ ID NO: 23 having a (GGGGS)₂+GGGG (SEQ ID NO: 121) linker between the repeats has an amino acid sequence of SEQ ID NO: 131.

As an example for DSP105 comprising such a 3 repeats 4-1BBL amino acid sequence, models of DSP105_V31 (SEQ ID NO: 147) were generated using homology modeling followed by side chains and loop refinement. Homology modeling was performed for each part based on a homologue X-ray structure. For PD1 – PDB IDs: 3RRQ, 5GGR, 5GGS, 5JXE and 4ZQK were used as templates. For hIgG4 – PDB IDs: 4C54, 4C55, 5W5M and 5W5N were used as templates. For 41BB-L – PDB IDs: 6CPR, 6A3V and 6CU0 were used as templates. Linker segments were modeled using loop modeling in CHARMM primarily in order to remove structural violations and to enable a plausible estimation for a possible 'spacer'. These models analysis predicted possible binding to the ligands and no interference between the different domains (Figures 13A-D).

Furthermore, a PD1-4-1BBL fusion protein comprising a (GGGGS)₂ (SEQ ID NO: 149) linker has been designed, to allow longer distance between PD1 moieties of distinct PD1-4-1BBL fusion proteins following trimerization.

Following, several PD1-4-1BBL variants were designed. Their sequences including the rationale for their selection and their 3D model are demonstrated in Table 4 hereinbelow and Figures 4-6.

Table 4: Description of the designed PD1-4-1BBL variants

Variant	Sequence	description		comment
DSP105	PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSN TSESVLNWYRMSPSNQTDKLAAPEDRSQPGQDCRF RVTLQPNGRDFHMSVVRARRNDSTGLYLCGAISLAPKA QIKESLRAELRVTTERRAEVPTAHPSPPRPAQQFQTLVG ACPWAVSGARASPGSAAAPRLREGPELSPDDPAGLLDL RQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGL SYKEDTKELVVAKAGVYVFFQLELRVVAEGEGSV SLALHLQPLRSAAGAAALATVDLPPASSEARNSAFGF QGRLLHLSAQRLGVHLHTEARARHAWQLTQGATVL GLFRVTPEIPAGLPSRSE (SEQ ID NO: 5)	PD1 SEQ ID NO: 2 (150 amino acids), glycine linker, 4-1BBL SEQ ID NO: 3 (205 amino acids)		
DSP105_ var1	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESV LNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRTQLP NGRDFHMSVVRARRNDSTGLYLCGAISLAPKAQIKESL RAELRVTTERRAEVPTAHPSPPRPAQQGSAAAPRLREG PELSPDDPAGLLDLRQGMFAQLVAQNVLIDGPLSWY SDPGLAGVSLTGGLSYKEDTKELVVAKAGVYVFFQLELRV VVAEGEGSVSLALHLQPLRSAAGAAALATVDLPPASSEARN SAFGFQGRLLHLSAQRLGVHLHTEARARHAWQLTQGATVL GLFRVTPEIPAGLPSRSE (SEQ ID NO: 12)	PD1 SEQ ID NO: 18 (140 amino acids), glycine linker, 4-1BBL SEQ ID NO: 22 (191 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 5AA from the C-ter of PD1 + 14AA from the N-ter of 4-1BBL (keeping the Gly linker)	Removing small hydrophobic flanking segments (predicted to be less structured) from both terminals of PD1, in addition to removal of the first 14 N- ter AA of 4-1BBL due to the long unstructured and hydrophobic segments preceding the structured domain of 4- 1BBL
DSP105_ var2	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESV LNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRTQLP NGRDFHMSVVRARRNDSTGLYLCGAISLAPKAQIKESL RAELRVTTERRAEVPTAHPSPPRPAQQGARASPGSAA PRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLID GPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGV YVFFQLELRVVAEGEGSVSLALHLQPLRSAAGAA ALATVDLPPASSEARNSAFGFQGRLLHLSAQRLGV HLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSR SE (SEQ ID NO: 14)	PD1 SEQ ID NO: 18 (140 amino acids), glycine linker, 4-1BBL SEQ ID NO: 23 (197 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 5AA from the C-ter of PD1 + 8AA from the N- ter of 4-1BBL (keeping the Gly linker)	Removing small hydrophobic flanking segments (predicted to be less structured) from both terminals of PD1, in addition to removal of the first 8 N- ter AA of 4-1BBL which are predicted to be unstructured and are the core hydrophobic segment preceding the structured domain of 4-1BBL

DSP105_ var3	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESV LNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLP NGRDFHMSVVRARRNDSTYLCAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPRPAGQGLREGPELSPD DPAGLLDLRQGMFAQLVAQNVLIDGPLSWSDPGLA GVSLTGGLSYKEDTKELVAKAGVYVFFQLELRRVV AGEGSGVSLALHLQPLRSAAGAAALATVLDLPPASSE ARNSAFGQGRLLHLSAGQRLGVHLHTEARARHAWQ LTQGATVGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 16)	PD1 SEQ ID NO: 18 (140 amino acids), glycine linker, 4-IBBL SEQ ID NO: 24 (185 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 5AA from the C-ter of PD1 + 20AA from the N-ter of 4-IBBL (keeping the Gly linker)	Removing small hydrophobic flanking segments (predicted to be less structured) from both terminals of PD1, in addition to removal of the first 20 N- ter AA of 4-IBBL due to the long unstructured and hydrophobic segments preceding the structured domain of 4- IBBL
DSP105_ var4	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESV LNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLP NGRDFHMSVVRARRNDSTYLCAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPRPAGQGSARASPGSA ASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLII DGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAG VYVFFQLELRRVVAGEGSGVSLALHLQPLRSAAGA AALATVLDLPPASSEARNSAFGQGRLLHLSAGQRLG VHLHTEARARHAWQLTQGATVGLFRVTPEIPAGLPSP RSE (SEQ ID NO: 44)	PD1 SEQ ID NO: 18 (140 amino acids), glycine linker, 4-IBBL SEQ ID NO: 25 (199 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 5AA from the C-ter of PD1 + Deletion of 6 amino acids from the N- terminal of 4-IBBL	Removing small hydrophobic flanking segments (predicted to be less structured) from both terminals of PD1, and Removing core hydrophobic segment of 4-IBBL
DSP105_ var5	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESV LNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLP NGRDFHMSVVRARRNDSTYLCAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPRPAGQGSARASPRLRE GPELSPDDPAGLLDLRQGMFAQLVAQNVLIDGPLSW YSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYVFFQ LELRRVVAGEGSGVSLALHLQPLRSAAGAAALATV DLPPASSEARNSAFGQGRLLHLSAGQRLGVHLHTEAR ARHAWQLTQGATVGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 45)	PD1 SEQ ID NO: 21 (133 aa), glycine linker, 4- IBBL SEQ ID NO: 25 (199 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 12AA from the C-ter of PD1 + Deletion of 6 amino acids from the N- terminal of 4-IBBL	Removing a small hydrophobic segment from N-ter of PD1 and a larger flanking segment (predicted to be less structured) from the C-ter of PD1 (based on crystalized constructs), in addition to removing core hydrophobic segment of 4-IBBL

DSP105_{var6}	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFV LNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLP NGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPGSAASPRLEGPESLPPD PAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAG VSLTGGLSYKEDTKELVVAAGVYVFFQLELRRVVA GEGSGVSLALHLQPLRSAAGAAALATVLDLPPASSE RNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQL TQGATV/LGLFRVTPEIPAGLPSRSE (SEQ ID NO: 46)	PD1 SEQ ID NO: 21 (133 aa), glycine linker, 4- IBBL SEQ ID NO: 22 (191 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 12AA from the C-ter of PD1 + Deletion of 14 amino acids from the N- terminal of 4-IBBL	Removing a small hydrophobic segment from N-ter of PD1 and a larger flanking segment (predicted to be less structured) from the C-ter of PD1 (based on crystalized constructs), in addition to removing flanking (predicted to be less structured) and hydrophobic segment of 4-IBBL
DSP105_{var7}	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFV LNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLP NGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPGARASPGSAASPRLEGP ELSPDDPAGLLDLRQGMFAQLVAQNVLIDGPLSWYS DPGLAGVSLTGGLSYKEDTKELVVAAGVYVFFQLE LRRVVAEGSGSVSLALHLQPLRSAAGAAALATVLDL PPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARA RHAWQLTQGATV/LGLFRVTPEIPAGLPSRSE (SEQ ID NO: 47)	PD1 SEQ ID NO: 21 (133 aa), glycine linker, 4- IBBL SEQ ID NO: 23 (197 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 12AA from the C-ter of PD1 + Deletion of 8 amino acids from the N- terminal of 4-IBBL	Removing a small hydrophobic segment from N-ter of PD1 and a larger flanking segment (predicted to be less structured) from the C-ter of PD1 (based on crystalized constructs), in addition to removal of the first 8 N-ter AA of 4-IBBL which are predicted to be unstructured and are the core hydrophobic segment preceding the structured domain of 4-IBBL
DSP105_{var8}	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFV LNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLP NGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPGLREGPELSPDDPAGLLD LRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGG LSYKEDTKELVVAAGVYVFFQLELRRVVAEGSGS VSLALHLQPLRSAAGAAALATVLDLPPASSEARNSAFG FQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATV LGLFRVTPEIPAGLPSRSE (SEQ ID NO: 48)	PD1 SEQ ID NO: 21 (133 aa), glycine linker, 4- IBBL SEQ ID NO: 24 (185 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 12AA from the C-ter of PD1 +20AA from the N- ter of 4-IBBL (keeping the Gly linker)	Removing a small hydrophobic segment from N-ter of PD1 and a larger flanking segment (predicted to be less structured) from the C-ter of PD1 (based on crystalized constructs), in addition to removal of the first 20 N-ter AA of 4-IBBL due to the long unstructured and hydrophobic segments preceding the structured domain of 4- IBBL

DSP105_{var9}	PWNPTTFSPALLVVTEDGNATFTCSFSNTSESFVLNWW RMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRD FHMSVVRARRNDSTGLYLCGALSAPKQAQIKESLRAELR VTERRAEVPTAHPSPGSGARASPGSAASPRRLREGPELSP DDPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGL AGVSLTGGLSYKEDTKELVVAAGVYVFFQLELRRV VAGEGSGVSLALHLQPLRSAAGAAALATVDLPPASS EARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAW QLTQGATVGLFRVTPEIPAGLPSRSE (SEQ ID NO: 49)	PD1 SEQ ID NO: 19 (128 aa), glycine linker, 4- IBBL SEQ ID NO: 25 (199 amino acids)	Deletion of the 10AA from the N-ter of PD1 + 12AA from the C-ter of PD1 + Deletion of 6 amino acids from the N- terminal of 4-IBBL	Removing larger flanking segments (predicted to be less structured) from both terminals of PD1 (based on crystallized constructs), in addition to removing core hydrophobic segment of 4-IBBL
DSP105_{var10}	PWNPTTFSPALLVVTEDGNATFTCSFSNTSESFVLNWW RMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRD FHMSVVRARRNDSTGLYLCGALSAPKQAQIKESLRAELR VTERRAEVPTAHPSPGsAASPRRLREGPELSPDDPAGLLD LRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGG LSYKEDTKELVVAAGVYVFFQLELRRVVAEGSGS VSLALHLQPLRSAAGAAALATVDLPPASSEARNSAFG FQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATV LGLFRVTPEIPAGLPSRSE (SEQ ID NO: 50)	PD1 SEQ ID NO: 19 (128 aa), glycine linker, 4- IBBL SEQ ID NO: 22 (191 amino acids)	Deletion of the 10AA from the N-ter of PD1 + 12AA from the C-ter of PD1 + Deletion of 14 amino acids from the N- terminal of 4-IBBL	Removing larger flanking segments (predicted to be less structured) from both terminals of PD1 (based on crystallized constructs), in addition to in addition to removing flanking (predicted to be less structured) and hydrophobic segment of 4-IBBL
DSP105_{var11}	PWNPTTFSPALLVVTEDGNATFTCSFSNTSESFVLNWW RMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRD FHMSVVRARRNDSTGLYLCGALSAPKQAQIKESLRAELR VTERRAEVPTAHPSPGARASPGSAASPRRLREGPELSPD DPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLA GVSLTGGLSYKEDTKELVVAAGVYVFFQLELRRV AGEGSGVSLALHLQPLRSAAGAAALATVDLPPASSE ARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQ LTQGATVGLFRVTPEIPAGLPSRSE (SEQ ID NO: 51)	PD1 SEQ ID NO: 19 (128 aa), glycine linker, 4- IBBL SEQ ID NO: 23 (197 amino acids)	Deletion of the 10AA from the N-ter of PD1 + 12AA from the C-ter of PD1 + Deletion of 8 amino acids from the N- terminal of 4-IBBL	Removing larger flanking segments (predicted to be less structured) from both terminals of PD1 (based on crystallized constructs), in addition to removal of the first 8 N-ter AA of 4- IBBL which are predicted to be unstructured and are the core hydrophobic segment preceding the structured domain of 4-IBBL
DSP105_{var12}	PWNPTTFSPALLVVTEDGNATFTCSFSNTSESFVLNWW RMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRD FHMSVVRARRNDSTGLYLCGALSAPKQAQIKESLRAELR VTERRAEVPTAHPSPGRLREGPELSPDDPAGLLDLRQGM FAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGLSYKE DTKELVVAAGVYVFFQLELRRVVAEGSGSVSLAL HLQPLRSAAGAAALATVDLPPASSEARNSAFGFQGR LLHLSAGQRLGVHLHTEARARHAWQLTQGATVGLFL RVTPEIPAGLPSRSE (SEQ ID NO: 52)	PD1 SEQ ID NO: 19 (128 aa), glycine linker, 4- IBBL SEQ ID NO: 24 (185 amino acids)	Deletion of the 10AA from the N-ter of PD1 + 12AA from the C-ter of PD1 +20AA from the N- ter of 4-IBBL (keeping the Gly linker)	Removing larger flanking segments (predicted to be less structured) from both terminals of PD1 (based on crystallized constructs), in addition to removal of the first 20 N-ter AA of 4- IBBL due to the long unstructured and hydrophobic segments preceding the structured domain of 4-IBBL

DSP105₋ var13	PWNPTTFSPALLVVTEGDNATFTCSFSNTSEFVLNWY RMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRD FHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELR VTERRAEVPTAHPSPPRPAQQGSGARASPGSAASPRL REGPELSPDDPAGLLDLRQQMFAQLVAQNVLLIDGPLS WYSDPGLAGVSLTGGLSYKEDTKELVVAAGVYYVF FQLELRRVVAGEGSGSVSLALHLQPLRSAGAAALAL TVDLPPASSEARNSAFGFQGRLLHLHSAGQRLGVHLHTE ARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSE (SEQ ID NO: 53)	PD1 SEQ ID NO: 20 (135 amino acids), glycine linker, 4-IBBL SEQ ID NO: 25 (199 amino acids)	Deletion of the 10AA from the N-ter of PD1 + 5AA from the C-ter of PD1 + Deletion of 6 amino acids from the N- terminal of 4-IBBL	Removing larger flanking segment (predicted to be less structured) from N-ter and smaller hydrophobic segment from C-ter of PD1 (based on crystallized constructs), in addition to removing core hydrophobic segment of 4-IBBL
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DSP105₋ var14	PWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWY RMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRD FHMSVVRRNRNDSGTLYLCGAISLAPKAQIKESLRAELR VTERRAEVPTAHPSPPRPAQGSAASPRLREGPELSPD DPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLA GVSLTGGLSYKEDTKELVVAKAGVYVFFQLELRRVV AGEGSGSVSLALHLQPLRSAAGAAALATVDLPPASSE ARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQ LTQGATVVLGLFRVTPPEIPAGLPSPRSE (SEQ ID NO: 54)	PD1 SEQ ID NO: 20 (135 amino acids), glycine linker, 4-1BBL SEQ ID NO: 22 (191 amino acids)	Deletion of the 10AA from the N-ter of PD1 + 5AA from the C-ter of PD1 + Deletion of 14 amino acids from the N- terminal of 4-1BBL	Removing larger flanking segment (predicted to be less structured) from N-ter and smaller hydrophobic segment from C-ter of PD1 (based on crystalized constructs), in addition to removing flanking (predicted to be less structured) and hydrophobic segment of 4-1BBL
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DSP105 – var15	PWNPTTFSPALLVVTEGDNATFTCSFSNTSEFVLNWWY RMSPSNQTDKLA AFPEDRSQPGQDCRFRVTQLPNGRD FHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELR VTERRAEVPTAHPSPRPAGOGARASPGSAA SPRLRE GPELSPDDPAGLLDLRQGMFAQLV AQNVLIDGPLSW YSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYVFFQ LELRRV VAGEGSGSVSLALHLQPLRSAAAGAAALATV DLPPASSEARNSAFGFQGRLLHLHSAGQRLGVHLHTEAR ARHAWQLTQGTATV LGLFRVTPEIPAGLPSRSE (SEQ ID NO: 55)	PD1 SEQ ID NO: 20 (135 amino acids), glycine linker, 4-1BBL SEQ ID NO: 23 (197 amino acids)	Deletion of the 10AA from the N-ter of PD1 + 5AA from the C-ter of PD1 + Deletion of 8 amino acids from the N- terminal of 4-1BBL	Removing a larger flanking segment (predicted to be less structured) from N-ter and a smaller hydrophobic segment from C-ter of PD1 (based on crystalized constructs), in addition to removal of the first 8 N-ter AA of 4- 1BBL which are predicted to be unstructured and are the core hydrophobic segment preceding the structured domain of 4-1BBL
DSP105 – var16	PWNPTTFSPALLVVTEGDNATFTCSFSNTSEFVLNWWY RMSPSNQTDKLA AFPEDRSQPGQDCRFRVTQLPNGRD FHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELR VTERRAEVPTAHPSPRPAGQGLREGPELSPDDPAGL LDLRQGMFAQLV AQNVLIDGPLSWYSDPGLAGVSLT GGLSYKEDTKELV VAKAGVYVFFQLELRRVVAGEG SGSVSLALHLQPLRSAAAGAAALATVDLPPASSEARN SAFGFQGRLLHLHSAGQRLGVHLHTEARARHAWQLTQ ATV LGLFRVTPEIPAGLPSRSE (SEQ ID NO: 56)	PD1 SEQ ID NO: 20 (135 amino acids), glycine linker, 4-1BBL SEQ ID NO: 24 (185 amino acids)	Deletion of the 10AA from the N-ter of PD1 + 5AA from the C-ter of PD1 + 20AA from the N-ter of 4-1BBL (keeping the Gly linker)	Removing a larger flanking segment (predicted to be less structured) from N-ter and a smaller hydrophobic segment from C-ter of PD1 (based on crystalized constructs), in addition to removal of the first 20 N-ter AA of 4- 1BBL due to the long unstructured and hydrophobic segments preceding the structured domain of 4-1BBL

DSP105_ var17	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFV LNWYRMSPSNQTDKLAAPEDRSQPGQDSRFRVTQLP NGRDFHMSVVRARRNDSTYLCGALSAPKAQIKESL RAELRVTERAEVPTAHPSPRPAGQGSAASPRRLREG PELSPDDPAGLLDLRQGMFAQLVAQNVLIDGPLSWY SDPGLAGVSLTGGLSYKEDTKELVVAKAGVYVFFQL ELRRVVAEGSGSVSLALHLQPLRSAAGAAALALTVD LPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARA RHAWQLTQGATVGLGLFRVTPEIPAGLPSRSE (SEQ ID NO: 97)	PD1 SEQ ID NO: 75 (140 amino acids), glycine linker, 4-1BBL SEQ ID NO: 22 (191 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 5AA from the C-ter of PD1 + Mutation of C73 corresponding to SEQ ID NO: 2 to S + Deletion of the N-ter segment from 4-1BBL 14AA	Removing a small hydrophobic segment from N-ter of PD1 and a larger flanking segment (predicted to be less structured) from the C-ter of PD1 (based on crystalized constructs); a mutation of C to S in PD1 to prevent un- desired S-S bridges; and removing a flanking (predicted to be less structured) and hydrophobic segment of 4-1BBL
DSP105_ var18	WNPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYR MSPSNQTDKLAAPEDRSQPGQDSRFRVTQLPNGRDF HMSVVRARRNDSTYLCGALSAPKAQIKESLRAELRV TERAEVPTAHPSPGSAASPRRLREGPELSPDDPAGLLDL RQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGL SYKEDTKELVVAKAGVYVFFQLLELRRVVAEGSGSV SLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGF QGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVL GLFRVTPEIPAGLPSRSE (SEQ ID NO: 101)	PD1 SEQ ID NO: 87 (127 amino acids), glycine linker, 4-1BBL SEQ ID NO: 22 (191 amino acids)	Deletion of the 11AA from the N-ter of PD1 + 12AA from the C-ter of PD1 + Mutation of C73 corresponding to SEQ ID NO: 2 to S + Deletion of the N-ter segment from 4-1BBL 14AA	Removing a larger flanking segment (predicted to be less structured) from N-ter and a large hydrophobic segment from C-ter of PD1 (based on crystalized constructs); a mutation of C to S in PD1 to prevent un desired S-S bridges; and removing a flanking (predicted to be less structured) and hydrophobic segment of 4-1BBL
DSP105_ var19	WNPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYR MSPSNQTDKLAAPEDRSQPGQDSRFRVTQLPNGRDF HMSVVRARRNDSTYLCGALSAPKAQIKESLRAELRV TERAEVPTAHPSPRPAGQGSAASPRRLREGPELSPDD PAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAG VSLTGGLSYKEDTKELVVAKAGVYVFFQLLELRRVVA GEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSE ARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQL TQGATVGLGLFRVTPEIPAGLPSRSE (SEQ ID NO: 103)	PD1 SEQ ID NO: 91 (134 amino acids), glycine linker, 4-1BBL SEQ ID NO: 22 (191 amino acids)	Deletion of the 11AA from the N-ter of PD1 + 5AA from the C-ter of PD1 + Mutation of C73 corresponding to SEQ ID NO: 2 to S + Deletion of the N-ter segment from 4-1BBL 14AA	Removing a larger flanking segment (predicted to be less structured) from N-ter and a small hydrophobic segment from C-ter of PD1 (based on crystalized constructs), a mutation of C to S in PD1 to prevent un desired S-S bridges; and removing flanking (predicted to be less structured) and hydrophobic segment of 4-1BBL

DSP105_ var20	DSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFV LNWYRMSPSNQTDKLAAPEDRSQPGQDSRFRVTQLP NGRDFHMSVVRARRNDSTGTYLCAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPGSAASPRLREGPELSPDDP AGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAG VSLTGGLSYKEDTKELVAKAGVYVFFQLELRVVA GEGSGSVSLALHLQPLRSAGAAALALTVDLPPASSEA RNSAFGFGQRLHLHLSAGQRLGVHLHTEARARHAWQL TQGATVGLGFRVTPEIPAGLPSRSE (SEQ ID NO: 105)	PD1 SEQ ID NO: 83 (133 amino acids), glycine linker, 4-1BBL SEQ ID NO: 22 (191 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 12AA from the C-ter of PD1 + Mutation of C73 corresponding to SEQ ID NO: 2 to S + Deletion of the N-ter segment from 4-1BBL 14AA	Removing a small flanking segment (predicted to be less structured) from N-ter and a large hydrophobic segment from C-ter of PD1 (based on crystalized constructs); a mutation of C to S in PD1 to prevent un desired S-S bridges; and removing a flanking (predicted to be less structured) and hydrophobic segment of 4-1BBL
DSP105_ var21	DSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFV LNWYRMSPSNQTDKLAAPEDRSQPGQDSRFRVTQLP NGRDFHMSVVRARRNDSTGTYLCAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPRPAAGSAASPRLREGPEL SPDDPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDP GLAGVSLTGGLSYKEDTKELVAKAGVYVFFQLELR RVVAGEGSGSVSLALHLQPLRSAGAAALALTVDLPP ASSEARNSAFGFGQRLHLHLSAGQRLGVHLHTEARARH AWQLTQGATVGLGFRVTPEIPAGLPSRSE (SEQ ID NO: 107)	PD1 SEQ ID NO: 95 (138 amino acids), glycine linker, 4-1BBL SEQ ID NO: 22 (191 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 7AA from the C-ter of PD1 + Mutation of C73 corresponding to SEQ ID NO: 2 to S + Deletion of the N-ter segment from 4-1BBL 14AA	Removing a larger flanking segment (predicted to be less structured) from N-ter and a small hydrophobic segment from C-ter of PD1 (based on crystalized constructs); a mutation of C to S in PD1 to prevent un desired S-S bridges; and removing flanking (predicted to be less structured) and hydrophobic segment of 4-1BBL
DSP105_ var22	PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSN TSEFV/LNWYRMSPSNQTDKLAAPEDRSQPGQDSRF RVTQLPNGRDFHMSVVRARRNDSTGTYLCAISLAPKA QIKESLRAELRVTERRAEVPTAHPSPRPAAGQFQTLVG ACPWAVSGARASPGSAASPRLREGPELSPDDPAGLLDL RQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGL SYKEDTKELVAKAGVYVFFQLELRVVAAGEGSGSV SLALHLQPLRSAGAAALALTVDLPPASSEARNSAFGF QGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVL GLFRVTPEIPAGLPSRSE (SEQ ID NO: 111)	PD1 SEQ ID NO: 73 (156 amino acids), glycine linker, 4-1BBL SEQ ID NO: 3 (205 amino acids)	Mutation of C73 corresponding to SEQ ID NO: 2 to S	A mutation of C to S in PD1 to prevent un desired S-S bridges

PCT/IL2019/050782

DSP105_ var23	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESV LNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLP NGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPRPAAGSAA SPRLREGPEL SPDDPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDP GLAGVSLTGGLSYKEDTKELVAKAGVYVFFQLELR RVVAGEGSGSVSLALHLQPLRSAGAAALATVDLPP ASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARAH AWQLTQGATVGLFRVTPEIPAGLPSRSE (SEQ ID NO: 113)	PD1 SEQ ID NO: 93 (138 amino acids), glycine linker, 4-1BBL SEQ ID NO: 22 (191 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 7AA from the C-ter of PD1 Deletion of the N- ter segment from 4- 1BBL 14AA	Removing a larger flanking segment (predicted to be less structured) from N-ter and a small hydrophobic segment from C-ter of PD1 (based on crystalized constructs) and removing flanking (predicted to be less structured) and hydrophobic segment of 4-1BBL
DSP105_ var24	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESV LNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLP NGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPRPGREGPELSPDDPAGL LDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLT GGLSYKEDTKELVAKAGVYVFFQLELRVAVAGEG SGSVSLALHLQPLRSAGAAALATVDLPPASSEARN AFGFQGRLLHLSAGQRLGVHLHTEARAHAWQLTQG ATVGLFRVTPEIPAGLPSRSE (SEQ ID NO:133)	PD1 SEQ ID NO: 115 (136 amino acids), glycine linker, 4-1BBL SEQ ID NO: 123 (184 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 9AA from the C-ter of PD1. Deletion of 21AA From the N-ter segment of 4-1BBL	shortening the relatively long linker (from both PD1 C-ter side and 41BBL N-ter side) keeping maximal positive charged residues to complement for the negatively charged N-ter of 4-1BBL
DSP105_ var25	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESV LNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLP NGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPRPGPELSPDDPAGLLD LRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGG LSYKEDTKELVAKAGVYVFFQLELRVAVAGEGSGS VSLALHLQPLRSAGAAALATVDLPPASSEARNSAFG FQGRLLHLSAGQRLGVHLHTEARAHAWQLTQGATV LGLFRVTPEIPAGLPSRSE (SEQ ID NO:135)	PD1 SEQ ID NO: 115 (136 amino acids), glycine linker, 4-1BBL SEQ ID NO: 125 (182 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 9AA from the C-ter of PD1. Deletion of 23AA From the N-ter segment of 4-1BBL	Larger shortening of the relatively long linker (from both PD1 C-ter side and 41BBL N-ter side), keeping maximal positive charged residues to complement for the negatively charged N-ter of 41BBL

DSP105 var26	PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSN TSESVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRF RVTLQPNGRDFHMSVVRARRNDSTYLCGALSAPKA QIKESLRAELRVTTERRAEVPTAHPSPPRPAQGSAAASP RLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLIDG PLSWYSDPGLAGVSLTGGLSYKEDTKELVVAAGVY YVFFQLELRRVVAEGSGSVSLALHLQPLRSAAGAAA LALTVDLPPASSEARNSAFGFQGRLLHLSAQRLGVHL HTEARARHAWQLTQGATVGLFRVTPEIPA (SEQ ID NO:137)	PD1 SEQ ID NO: 117 (145 amino acids), glycine linker, 4-1BBL SEQ ID NO: 127 (183 amino acids)	Deletion of the 5AA from the C-ter of PD1. Deletion of 14 AA from the N-ter segment of 4-1BBL and 8 AA from the C-ter of 4-1BBL	No deletion of N-ter of PD1 (the N-ter is at the PDL1 interaction side, therefore, there is a possibility that deletion at this area may affect the proteins interaction). Removing hydrophobic segment from C-ter of PD1 (based on crystalized constructs) and removing flanking (predicted to be less structured) and hydrophobic segment of 4-1BBL and the C-ter side of 41BBL seq (possible interaction of this segment with the linker)
DSP105 var27	PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSN TSESVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRF RVTLQPNGRDFHMSVVRARRNDSTYLCGALSAPKA QIKESLRAELRVTTERRAEVPTAHPSPPRPAQREGPELS PDDPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPG LAGVSLTGGLSYKEDTKELVVAAGVYVFFQLELRR VVAEGSGSVSLALHLQPLRSAAGAAAALATVDLPPA SSEARNSAFGFQGRLLHLSAQRLGVHLHTEARARHIA WQLTQGATVGLFRVTPEIPA (SEQ ID NO:139)	PD1 SEQ ID NO: 119 (143 amino acids), glycine linker, 4-1BBL SEQ ID NO: 129 (176 amino acids)	Deletion of the 7AA from the C-ter of PD1. Deletion of 21 AA from the N-ter segment of 4-1BBL and 8 AA from the C-ter of 4-1BBL	Shortening the relatively long linker (from both PD1 C-ter side and 41BBL N-ter side) and the C-ter side of 41BBL seq (possible interaction of this segment with the linker)
DSP105 var28	DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESV LNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRTQLP NGRDFHMSVVRARRNDSTYLCGALSAPKAQIKESL RAELRVTTERRAEVPTAHPSPPRPAQGCAPWAVSGA RASPGSAAAPRLREGPELSPDDPAGLLDLRQGMFAQLV AQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL VVAAGVYVFFQLELRRVVAEGSGSVSLALHLQPL RSAAGAAAALATVDLPPASSEARNSAFGFQGRLLHLSA GQRLGVHLHTEARARHAWQLTQGATVGLFRVTPEIP AGLPSRSE (SEQ ID NO:141)	PD1 SEQ ID NO: 18 (140 amino acids), glycine linker, 4-1BBL SEQ ID NO: 3 (205 amino acids)	Deletion of the 5AA from the N-ter of PD1 + 5AA from the C-ter of PD1	Removing a small flanking segment (predicted to be less structured) from N-ter and a small hydrophobic segment from C-ter of PD1 (based on crystalized constructs). Living relatively long linker between the PD1 and 4-1BBL to allow long distance between the PD1 domains after trimerization of the 4- 1BBL

DSP105 var29	PGWFLDSPDRPWNPPTFSFALLVVTEGDNATFTCSFSN TSEFVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRF RVTQLPNGRDFHMSVVRARRNDSTYLCGALSAPKA QIKESLRAELRVTTERRAEVPTAHPSPPRPAQQFQTLVG SGARASPGSAAAPRLREGPELSPDDPAGLLDLRQGMFA QLVAQNVLIDGPLSWYSDPGLAGVSLTGGLSYKEDT KELVAKAGVYVFFQLELRVVAEGSGSVSLAHL QPLRSAAAGAAALATVDLPPASSEARNSAFGFQGRLLH LSAQRLGVHLHTEARARHAWQLTQGATVVLGLFRVT PEIPAGLPSRSE (SEQ ID NO:143)	PD1 SEQ ID NO: 2 (150 amino acids), glycine linker, 4-IBBL SEQ ID NO: 25 (109 amino acids)	Deletion of 6 AA from the N-ter segment of 4-IBBL	A long native sequence without defined structure which may serve as a long linker between the PD1 and 4-IBBL to allow long distance between the PD1 domains after trimerization of the 4- IBBL. Removing core hydrophobic segment from the 4-IBBL.
DSP105 var30	PGWFLDSPDRPWNPPTFSFALLVVTEGDNATFTCSFSN TSEFVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRF RVTQLPNGRDFHMSVVRARRNDSTYLCGALSAPKA QIKESLRAELRVTTERRAEVPTAHPSPPRPAQQFQTLVG GGSGGGGSACPWAVSGARASPGSAAAPRLREGPELS PDDPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPG LAGVSLTGGLSYKEDTKELVAKAGVYVFFQLELR VVAEGSGSVSLAHLQPLRSAAAGAAALATVDLPPA SSEARNSAFGFQGRLLHLSAQRLGVHLHTEARARHA WQLTQGATVVLGLFRVTPEIPAGLPSRSE (SEQ ID NO:145)	PD1 SEQ ID NO: 2 (150 amino acids), (GGGS)x2 (SEQ ID NO: 149) linker 4-IBBL SEQ ID NO: 3 (205 amino acids)	WT PD1 and 4-IBBL linked by (GGGS)x2	Extension of the linker between the PD1 and 4-IBBL to allow long distance between the PD1 domains after trimerization of the 4-IBBL

DSP105₋ var31	DSPDRPNPTTFSPALLVVTEGDNATFTCSFSNTSESV LNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLP NGRDFHMSVVRARRNDSTGLCGAISLAPKAQIKESL RAELRVTERAEVPTAHPSPRPAGQGSGGGSGGS AASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVL LIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKA GVVYVFFQLELRVVAAGSGSVSLALHLQPLRSAAG AAALATVDLPPASSEARNSAFGFQGRLLHLSAGQRL GVHLHTEARARHAWQLTQGATVGLGLFRVTPEIPAGLP SPRSEGGGGSGGGGGGSAASPRLREGPELSPDDPA GLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVS LTGGLSYKEDTKELVVAAGVYVFFQLELRVVAAGE GSGSVSLALHLQPLRSAAGAAALATVDLPPASSEARN SAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVGLFRVTPEIPAGLPSPRSEGGGGSGGGSGGG SAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNV LLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAK AGVYVFFQLELRVVAAGSGSVSLALHLQPLRSA GAAALATVDLPPASSEARNSAFGFQGRLLHLSAGQR LGVHLHTEARARHAWQLTQGATVGLGLFRVTPEIPAGL PSRSE (SEQ ID NO:147)	PD1 SEQ ID NO: 18 (140 amino acids),GGGGSGG GG (SEQ ID NO: 122) as a linker, sc3x4IBBL [SEQ ID NO:131 (601 aa): 3 times SEQ ID NO:22) separated by GGGGSGGGSGG GGG linker (SEQ ID NO:121)]	Deletion of the 5AA from the N-ter of PD1 + 5AA from the C-ter of PD1. Single chain containing three 4-IBBL with 14 AA deletion at the N-ter segment.	Removing a small flanking segment (predicted to be less structured) from N-ter and a small hydrophobic segment from C-ter of PD1 (based on crystalized constructs). Avoiding the need for trimerization by combining three domains of 4-IBBL (without the 14 AA of the N-ter which predicted to be less structured and hydrophobic)
DSP105₋ var32	SAA SPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNV LLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAK AGVYVFFQLELRVVAAGSGSVSLALHLQPLRSA GAAALATVDLPPASSEARNSAFGFQGRLLHLSAGQR LGVHLHTEARARHAWQLTQGATVGLGLFRVTPEIPAGL PSRSEGGSDPRPNPTTFSPALLVVTEGDNATFTCSFS NTSESVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCR FRVTQLPNGRDFHMSVVRARRNDSTGLCGAISLAPK AQIKESLRAELRVTERAEVPTAHPSPRPAGQ (SEQ ID NO:168)	4-IBBL SEQ ID NO:22 (191 amino acids), glycine linker, PD1 SEQ ID NO: 18 (140 amino acids)	Deletion of 14AA from the N-ter of 4-IBBL + the 5AA from the N-ter of PD1 + 5AA from the C-ter of PD1 (keeping the Gly linker)	Removing the first 14 N-ter AA of 4- IBBL due to the long unstructured and hydrophobic segments preceding the structured domain of 4-IBBL; and removing small hydrophobic flanking segments (predicted to be less structured) from both terminals of PD1

EXAMPLE 2**MANUFACTURING OF PD1-4-1BBL VARIANTS**

For comparative functional analysis and production evaluation, several PD1-4-1BBL fusion proteins were produced, namely: a PD1-4-1BBL fusion protein referred to herein as "DSP105" (SEQ ID NO: 5) and nine DSP105 variants referred to herein as "DSP105_V1", "DSP105_V2", "DSP105_V3", "DSP105_V17", "DSP105_V18", "DSP105_V19", "DSP105_V20", "DSP105_V21", "DSP105_V22", "DSP105_V23", "DSP105_V24", "DSP105_V25", "DSP105_V26", "DSP105_V27", "DSP105_V28", "DSP105_V29", "DSP105_V30" and "DSP105_V31" (SEQ ID NO: 12, 14, 16, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143, 145 and 147) respectively.

Production was effected in ExpiCHO, Expi293F or CHO-3E7 cells were transfected by a pcDNA3.4 or pTT5 expression vector cloned with coding sequence for DSP105 and its variants. The sequences were cloned into the vector using EcoRI and HindIII restriction enzymes, with addition of Kozak sequence, artificial signal peptide (MEAPAQLLFLLLLWLPDTTG, SEQ ID NO: 4) with or without 6 His-tag in the N or C terminus and a stop codon in the C terminus. The proteins were collected from the supernatant of cell culture,

The supernatants were purified by one-step purification by HisTrapTM FF Crude column.

The production of the tagged fusion proteins was verified by SDS-PAGE and Western blot analysis using specific antibodies against His tag or one of the domains of the molecule (i.e. the extracellular domain each of PD1 or 4-1BBL), see e.g. Figures 8A-E.

For examples, western blot analysis of variants His-tagged-DSP105_V17, DSP105-V18, DSP105_V19, DSP105_V20 and DSP105_V23 separated on an SDS-PAGE gel under reducing conditions followed by immunoblotting with an anti-PD1 antibody or anti His-tag antibody demonstrated that the variants were expressed in CHO-3E7 cells.

Optionally, the produced fusion proteins are further purified by size exclusion chromatography to collect the fraction of a purified trimer. To this end, the proteins are loaded on Superdex column with PBS as mobile phase and at a flow rate of 1ml/min. The major peak corresponding to protein trimers is collected.

Yield is assessed using BCA or Bradford protein concentration determination, or by optical density measurement by Nanodrop, at 280nm, divided by the protein extinction coefficient. Proteins stability is evaluated under storage and accelerated conditions.

EXAMPLE 3**DETERMINATION OF THE OLIGOMERIC STATE OF THE PD1-4-1BBL
VARIANTS**

Materials – N-terminal his-tagged DSP105 (SEQ ID NO: 1) produced in Expi293 cells
 5 [referred to herein as “DSP105 (HEK-NH)”] as described in Example 2 hereinabove, N-terminal
 his-tagged DSP105 (SEQ ID NO: 1) produced in ExpiCHO cells [referred to herein as “DSP105
 (CHO-NH)”], N-terminal his-tagged DSP105 variants DSP105_V1 (SEQ ID NO: 13), DSP105_V2
 (SEQ ID NO: 15) and DSP105_V3 (SEQ ID NO: 17) produced in ExpiCHO cells [referred to herein
 as “DSP105 (CHO-NH)-V1”, “DSP105 (CHO-NH)-V2” and “DSP105 (CHO-NH)-V3)], N-
 10 terminal His-tagged DSP105 variants DSP105_V17 (SEQ ID NO: 99) and DSP105_V22 (SEQ ID
 NO: 109) produced in CHO-3E7 or ExpiCHO cells and DSP105 variants DSP107_V17 (SEQ ID
 NO: 97), DSP105_V18 (SEQ ID NO: 101), DSP105_V19 (SEQ ID NO: 103), DSP105_V20 (SEQ
 ID NO: 105), DSP105_V21 (SEQ ID NO: 107), DSP_V22 (SEQ ID NO: 111), DSP105_V23 (SEQ
 ID NO: 113), DSP105_V24 (SEQ ID NO: 133), DSP105_V25 (SEQ ID NO: 135), DSP105_V26
 15 (SEQ ID NO: 137), DSP105_V27 (SEQ ID NO: 139), DSP105_V28 (SEQ ID NO: 141),
 DSP105_V29 (SEQ ID NO: 143), DSP105_V30 (SEQ ID NO: 145) and DSP105_V31 (SEQ ID
 NO: 147), produced in CHO-3E7 cells or ExpiCHO cells, as described in Example 2 hereinabove.

. Spectra BR Protein molecular weight marker (Thermo Fisher Scientific, cat# 26634), 4-20%
 polyacrylamide gel (BioRad, cat# 556-8094), e-Stain peds (GenScript, cat #L02011), Laemmli
 20 Loading buffer (BioRad, cat# 161-0747). Superdex 200 Increase column (GE Healthcare), SEC-
 MALS system: AKTA Explorer (GE) + MiniDawn TREOS + OPTILAB T-reX (WYATT).

Methods–

SDS-PAGE analysis - Two µg protein or 35 µl supernatant from each sample was mixed with
 loading buffer with or without β-mercaptoethanol (reduced and non-reduced conditions,
 25 respectively), heated for 5 minutes at 95 °C and separated on 4-20 % gradient polyacrylamide gel
 electrophoresis SDS-PAGE. Proteins migration on the gel is visualized by Coomassie blue staining
 with e-Stain peds and washing using the e-Stain machinery (GenScript), according to manufacturer
 instructions.

SEC-MALS analysis - Proteins are loaded on a Superdex 200 Increase column (GE
 30 Healthcare) and run at a flow rate of 0.8 ml / min with 10 mM KPO4 pH 8.0 + 150 mM NaCl as
 mobile phase. Detection is performed by UV, MALS and RI using AKTA Explorer (GE) +
 MiniDawn TREOS + OPTILAB T-reX (WYATT).

Results –

As demonstrated in Figure 7, a high proportion of high molecular weight forms (HMW, aggregates) was observed in N-terminal His-tagged DSP105 (SEQ ID NO: 1) under non-reducing conditions but not in reducing conditions. On the other hand, only a minor level of aggregates was detected in N-terminal His-tagged DSP105_V17 (SEQ ID NO: 99), indicating that aggregation of DSP105 due to incorrect disulfide bonds formation was prevented in DSP105_V17. A low level of aggregates was observed in other PD1-4-1BBL variants [namely, DSP105_V18 (SEQ ID NO: 101), DSP105_V19 (SEQ ID NO: 103), DSP105_V20 (SEQ ID NO: 105) and DSP105_V23 (SEQ ID NO: 113)], as demonstrated in Figures 8A-E.

As shown in Figures 9A-B, when separated on an SDS-PAGE under non-reducing conditions N-terminal his-tagged DSP105 (WT; SEQ ID NO: 1) His N-terminal His-tagged DSP105 variants DSP105_V17 (SEQ ID NO: 99), DSP105_V17 (SEQ ID NO: 97), DSP105_V20 (SEQ ID NO: 105), DSP_V22 (SEQ ID NO: 111), DSP105_V23 (SEQ ID NO: 113), DSP105_V24 (SEQ ID NO: 133), DSP105_V25 (SEQ ID NO: 135), and DSP105_V31 (SEQ ID NO: 147), variants of PD1-4-1BBL were detected at the same molecular weight as under reducing conditions. Additional bands of higher molecular weight were detected in his-tagged DSP105 (WT; SEQ ID NO: 1, designated as “DSP105WT” in Figures 9A-B), which were stronger under the non-reducing conditions compared to the reducing conditions. This might suggest the formation of multimers, probably mediated by S-S bridges. Interestingly this main “high molecular weight” band that appeared in non-reduced DSP105WT was not seen in DSP105_V22 (SEQ ID NO: 111) containing the CYS73>Ser substitution, emphasizing that this high molecular band is probably mediated by the CYS73.

EXAMPLE 4**THE PD1-4-1BBL VARIANTS CONTAIN BOTH DOMAINS**

Materials - DSP105 (HEK-NH) (SEQ ID NO: 1), DSP105 (CHO-NH) (SEQ ID NO: 1), DSP105 (CHO-NH)-V1 (SEQ ID NO: 13), DSP105 (CHO-NH)-V2 (SEQ ID NO: 15), DSP105 (CHO-NH)-V3 (SEQ ID NO: 17), N-terminal His-tagged DSP105_V17 (SEQ ID NO: 99), His-tagged DSP105_V22 (SEQ ID NO: 109), DSP_V17 (SEQ ID NO: 97), DSP105_V18 (SEQ ID NO: 101), DSP105_V19 (SEQ ID NO: 103), DSP105_V20 (SEQ ID NO: 105), DSP105_V21 (SEQ ID NO: 107), DSP_V22 (SEQ ID NO: 111), DSP105_V23 (SEQ ID NO: 113), DSP105_V24 (SEQ ID NO: 133), DSP105_V25 (SEQ ID NO: 135), DSP105_V26 (SEQ ID NO: 137), DSP105_V27 (SEQ ID NO: 139), DSP105_V28 (SEQ ID NO: 141), DSP105_V29 (SEQ

ID NO: 143), DSP105__V30 (SEQ ID NO: 145) and DSP105__V31 (SEQ ID NO: 147), produced as described in Example 2 hereinabove.

For the Western blot analysis: Spectra BR protein marker (Thermo Fisher Scientific, cat# 26634), Laemmli Loading buffer (BioRad, cat# 161-0747), 4-20% polyacrylamide gel (BioRad, cat# 556-8094), anti 4-1BBL (BioVision, 5369-100), anti PD1 (Cell Signaling, cat# 86163), anti-His (GenScript, Cat.No. A00186), secondary Goat Anti Rabbit IgG (H + L)-HRP Conjugate (R&D, cat# 170-6515), ECL Plus Western Blotting substrate (Pierce, cat# 32132).

For the sandwich ELISA: Anti 4-1BBL antibody (capture antibody from a matched pair; Abnova #H00008744-AP41), anti PD1-biotinylated antibody, Streptavidin Protein, HRP (# 21126, Thermo Scientific), TMB substrate (1-Step™ Ultra TMB-ELISA Substrate Solution, Thermo Scientific #34028).

Methods –

Western blot analysis - Proteins (50-500 ng per lane) were treated at reducing or non-reducing conditions (in loading buffer containing β -mercaptoethanol and boiled for 5 minutes at 95 °C, or, in sample buffer without β -mercaptoethanol without heating, respectively) and separated on a 4 – 20 % gradient SDS-PAGE gel. Following, proteins were transferred onto a PVDF membrane and incubated with primary antibodies overnight, anti 4-1BBL, anti-PD1 or anti-His, followed by 1 hour incubation with an HRP-conjugated secondary antibody. Signals were detected following ECL development.

Sandwich ELISA - Plates are coated with anti 4-1BBL capture antibody (2.5ug/ml in PBS) and blocked in blocking solution (PBS, 1 % BSA, 0.005 % Tween). The produced PD1-4-1BBL fusion proteins, serially diluted in blocking solution, are applied and incubated in coated plates for 2 hours, followed by incubation with detecting anti-PD1 biotinylated antibody, and subsequent detection with streptavidin-HRP and TMB substrate, according to manufacturer recommendation. Plates are analyzed using Plate reader (Thermo Scientific, Multiscan FC) at 450 nm, with reference at 620 nm.

EXAMPLE 5

THE PD1-4-1BBL VARIANTS BIND PDL1 AND 4-1BB

Binding analysis of the PD1 moiety of PD1-4-1BBL protein to PDL1

The binding of the PD1 domain of PD1-4-1BBL to human PDL1 was determined using DLD1-PDL1 cell line overexpressing PDL1. DLD1-WT cells served as a negative control. Cells were incubated with different concentrations of the produced PD1-4-1BBL fusion proteins,

followed by immuno-staining with a secondary anti 4-1BBL antibody. Binding was analyzed by flow cytometry.

Materials - DSP105 (HEK-NH) (SEQ ID NO: 1), DSP105 (CHO-NH) (SEQ ID NO: 1), DSP105 (CHO-NH)-V1 (SEQ ID NO: 13), DSP105 (CHO-NH)-V2 (SEQ ID NO: 15), DSP105 (CHO-NH)-V3 (SEQ ID NO: 17), N-terminal His-tagged DSP105_V17 (SEQ ID NO: 99), His-tagged DSP105_V22 (SEQ ID NO: 109), DSP105_V17 (SEQ ID NO: 97), DSP105_V18 (SEQ ID NO: 101), DSP105_V19 (SEQ ID NO: 103), DSP105_V20 (SEQ ID NO: 105), DSP105_V21 (SEQ ID NO: 107), DSP_V22 (SEQ ID NO: 111), DSP105_V23 (SEQ ID NO: 113), DSP105_V24 (SEQ ID NO: 133), DSP105_V25 (SEQ ID NO: 135), DSP105_V26 (SEQ ID NO: 137), DSP105_V27 (SEQ ID NO: 139), DSP105_V28 (SEQ ID NO: 141), DSP105_V29 (SEQ ID NO: 143), DSP105_V30 (SEQ ID NO: 145) and DSP105_V31 (SEQ ID NO: 147), produced as described in Example 2 hereinabove.

DLD1-WT and DLD1-PDL1 cell lines (Hendriks et al 2016), True stain FCX (Biolegend, cat#422302), anti 4-1BBL (Biolegend, cat#311506), isotype IgG1, k (Biolegend, cat#400122), anti PDL1 (Biolegend, cat#329708), isotype IgG2b, (Biolegend, cat#400322).

Methods - Cells were incubated for 30 minutes on ice with different concentrations (0.05-50 µg/ml) of the produced PD1-4-1BBL fusion proteins, followed by immuno-staining with antibodies against 4-1BBL and analysis by flow cytometry.

Binding analysis of the 4-1BBL moiety of PD1-4-1BBL protein to 4-1BB

The binding of the 4-1BBL domain of PD1-4-1BBL to human 4-1BB was determined using HT1080-4-1BB cell line overexpressing 4-1BB. HT1080 WT cells served as a negative control. Cells were incubated with different dilutions of the produced PD1-4-1BBL fusion proteins, followed by immuno-staining with a secondary anti PD1 antibody. Binding was analyzed by flow cytometry.

Materials - DSP105 (HEK-NH) (SEQ ID NO: 1), DSP105 (CHO-NH) (SEQ ID NO: 1), DSP105 (CHO-NH)-V1 (SEQ ID NO: 13), DSP105 (CHO-NH)-V2 (SEQ ID NO: 15), DSP105 (CHO-NH)-V3 (SEQ ID NO: 17), N-terminal His-tagged DSP105_V17 (SEQ ID NO: 99), His-tagged DSP105_V22 (SEQ ID NO: 109), DSP105_V17 (SEQ ID NO: 97), DSP105_V18 (SEQ ID NO: 101), DSP105_V19 (SEQ ID NO: 103), DSP105_V20 (SEQ ID NO: 105), DSP105_V21 (SEQ ID NO: 107), DSP_V22 (SEQ ID NO: 111), DSP105_V23 (SEQ ID NO: 113), DSP105_V24 (SEQ ID NO: 133), DSP105_V25 (SEQ ID NO: 135), DSP105_V26 (SEQ ID NO: 137), DSP105_V27 (SEQ ID NO: 139), DSP105_V28 (SEQ ID NO: 141), DSP105_V29 (SEQ ID NO: 143), DSP105_V30 (SEQ ID NO: 145) and DSP105_V31 (SEQ ID NO: 147), produced as described in Example 2 hereinabove.

HT1080 WT and HT1080-4-1BB cells (Wyzgol et al, 2009), Fixable Viability Dye (BD Biosciences, cat#562247), True stain FCX (Biolegend, cat#422302), anti PD1 (Biolegend cat#329908), anti 41BB (Biolegend cat#309801) isotype IgG1, K (Biolegend, cat# 400122).

Methods - Cells were incubated for 30 minutes on ice with different dilutions (1:8-1:1024) of the produced PD1-4-1BBL fusion proteins, followed by immuno-staining with antibodies against PD1 and analysis by flow cytometry.

Results - As shown in Figure 10A, DLD1-PD1 and HT1080-4-1BB cells express the relevant receptors, PD1 and 41BB, respectively. Binding assays showed that the His-tagged DSP105_V17 (SEQ ID NO: 97), binds to DLD1-PDL1 cells and to HT1080-41BB cells in a dose dependent manner (Figure 10B). Different dilutions of the supernatant control (from non-transfected cells) did not bind to HT1080-41BB cells (Figure 10B). Following, GMFI values were used to create a binding curve graph with a GraphPad Prism software (Figure 10C). Taken together, both sides of the His-tagged DSP105_V17 (SEQ ID NO: 97), fusion protein, bind their relevant counterparts overexpressed on the surface of cells.

Binding of PD1-4-1BBL to human, mouse and cynomolgus monkey PDL1 and 4-1BB counterparts

The binding of PD1-4-1BBL proteins to PDL1 and 4-1BB counterparts is determined by Surface Plasmon Resonance (SPR) assays.

Materials - DSP105 (HEK-NH) (SEQ ID NO: 1), DSP105 (CHO-NH) (SEQ ID NO: 1), DSP105 (CHO-NH)-V1 (SEQ ID NO: 13), DSP105 (CHO-NH)-V2 (SEQ ID NO: 15), DSP105 (CHO-NH)-V3 (SEQ ID NO: 17), N-terminal His-tagged DSP105_V17 (SEQ ID NO: 99), His-tagged DSP105_V22 (SEQ ID NO: 109), DSP105_V17 (SEQ ID NO: 97), DSP105_V18 (SEQ ID NO: 101), DSP105_V19 (SEQ ID NO: 103), DSP105_V20 (SEQ ID NO: 105), DSP105_V21 (SEQ ID NO: 107), DSP105_V22 (SEQ ID NO: 111), DSP105_V23 (SEQ ID NO: 113), DSP105_V24 (SEQ ID NO: 133), DSP105_V25 (SEQ ID NO: 135), DSP105_V26 (SEQ ID NO: 137), DSP105_V27 (SEQ ID NO: 139), DSP105_V28 (SEQ ID NO: 141), DSP105_V29 (SEQ ID NO: 143), DSP105_V30 (SEQ ID NO: 145) and DSP105_V31 (SEQ ID NO: 147), produced as described in Example 2 hereinabove.

Series S sensor chip CM5 (GE, cat. # BR100530), Ab capture kit, human hCD47 (negative control), human PDL1, mouse PDL1, cynomolgus PDL1, human 4-1BB, mouse 4-1BB, cynomolgus 4-1BB.

Methods - SPR assays are performed using Biacore T100 biosensor (GE Healthcare). Antibody from the capture kit is coupled to all four flow-channels of the chip (Fc1-4), using standard amine coupling protocol as recommended by the manufacturer. PD1-4-1BBL counterpart

proteins (PDL1 and 4-1BB) binding to the chip is performed in HBS-EP+ running buffer (10 mM HEPES pH7.3, 150 mM NaCl, 3 mM EDTA, 0.05 % Tween20): Human CD47 (negative control) is loaded onto the reference channel Fc1, while Fc2-4 are loaded with the human, mouse and cynomolgus PDL1 proteins. Following automated regeneration of the chip, the chip is re-loaded with the human CD47 on channel Fc1, and with the human, mouse and cynomolgus 4-1BB proteins on channels Fc2-4. Following counterparts binding, the PD1-4-1BBL analytes are passed over all four channels. This process is iteratively repeated with various concentrations of PD1-4-1BBL analytes at flow rate of 50 μ l / min. 3M MgCl₂ solution is injected (45 sec at 20 μ l/min) at the end of each cycle, to regenerate the active surface by dislodging the captured molecules. The binding parameters are evaluated using Kinetic 1:1 Binding model in BiaEvaluation software v. 3.0.2 (GE Healthcare).

EXAMPLE 6

DUAL SIDE BINDING – POTENCY ASSAY

The binding of both sides of the molecule to their counterparts, i.e. the binding of PD1 to PDL1 and the binding of 4-1BBL to 4-1BB, is tested by a sandwich ELISA based potency assay. This assay is also used to compare the functional properties of the different production batches.

Materials — DSP105 (HEK-NH) (SEQ ID NO: 1), DSP105 (CHO-NH) (SEQ ID NO: 1), DSP105 (CHO-NH)-V1 (SEQ ID NO: 13), DSP105 (CHO-NH)-V2 (SEQ ID NO: 15), DSP105 (CHO-NH)-V3 (SEQ ID NO: 17), N-terminal His-tagged DSP105_V17 (SEQ ID NO: 99), His-tagged DSP105_V22 (SEQ ID NO: 109), DSP105_V17 (SEQ ID NO: 97), DSP105_V18 (SEQ ID NO: 101), DSP105_V19 (SEQ ID NO: 103), DSP105_V20 (SEQ ID NO: 105), DSP105_V21 (SEQ ID NO: 107), DSP_V22 (SEQ ID NO: 111), DSP105_V23 (SEQ ID NO: 113), DSP105_V24 (SEQ ID NO: 133), DSP105_V25 (SEQ ID NO: 135), DSP105_V26 (SEQ ID NO: 137), DSP105_V27 (SEQ ID NO: 139), DSP105_V28 (SEQ ID NO: 141), DSP105_V29 (SEQ ID NO: 143), DSP105_V30 (SEQ ID NO: 145) and DSP105_V31 (SEQ ID NO: 147), produced as described in Example 2 hereinabove. Human recombinant PDL1 (ACRO Biosystem, A001-214), human recombinant protein 4-1BB-Biotin (cat#41B-H82E3, ACRO Biosystem), Streptavidin Protein-HRP (cat#21126, Thermoscientific), 1-Step™ Ultra TMB-ELISA Substrate Solution (cat#34028, Thermoscientific).

Methods - PDL1 is bound on the surface of a plastic plate, PD1-4-1BBL is added and allowed to bind to the immobilized PDL1. Following washing, biotinylated 4-1BB is added and allowed to bind to the 4-1BBL arm of the molecule. Following, the plates are washed and streptavidin HRP

is added. Detection is effected with a TMB substrate according to standard ELISA protocol using a Plate reader (Thermo Scientific, Multiscan FC) at 450 nm, with reference at 540 nm.

EXAMPLE 7

ACTIVATION OF 4-1BB BY THE PD1-4-1BBL VARIANTS

The activation effect of the 4-1BB receptor by the produced PD1-4-1BBL fusion proteins was determined using HT1080 cells or another cell line that are overexpressing the 4-1BB receptor. Specifically, the HT1080-4-1BB cell line is overexpressing 4-1BB and is known to secrete IL8 upon binding of 4-1BBL (Wyzgol et al., 2009, The Journal of Immunology). Upon binding of 4-1BBL to the 4-1BB receptor on the surface of these cells, a signaling pathway is activated resulting in secretion of IL8. To this end, the cells were incubated in the presence of different concentrations of the produced PD1-4-1BBL with or without PDL1 expressing cells, and IL8 secretion to the culture media was determined by ELISA.

Materials – DSP105 (HEK-NH) (SEQ ID NO: 1), DSP105 (CHO-NH) (SEQ ID NO: 1), DSP105 (CHO-NH)-V1 (SEQ ID NO: 13), DSP105 (CHO-NH)-V2 (SEQ ID NO: 15), DSP105 (CHO-NH)-V3 (SEQ ID NO: 17), N-terminal His-tagged DSP105_V17 (SEQ ID NO: 99), His-tagged DSP105_V22 (SEQ ID NO: 109), DSP105_V17 (SEQ ID NO: 99), DSP105_V18 (SEQ ID NO: 101), DSP105_V19 (SEQ ID NO: 103), DSP105_V20 (SEQ ID NO: 105), DSP105_V21 (SEQ ID NO: 107), DSP105_V22 (SEQ ID NO: 111), DSP105_V23 (SEQ ID NO: 113), DSP105_V24 (SEQ ID NO: 133), DSP105_V25 (SEQ ID NO: 135), DSP105_V26 (SEQ ID NO: 137), DSP105_V27 (SEQ ID NO: 139), DSP105_V28 (SEQ ID NO: 141), DSP105_V29 (SEQ ID NO: 143), DSP105_V30 (SEQ ID NO: 145) and DSP105_V31 (SEQ ID NO: 147), produced as described in Example 2 hereinabove.

HT1080-4-1BB cells, IL-8 ELISA kit (R&D, cat#D8000C), DMEM (Biological industries, cat# 01-055-1A), FBS (Rhenium, cat# 10270106), anti 4-1BB (Biolegend, cat#359810), isotype IgG1, k (Biolegend, cat#400122).

Methods - HT1080-4-1BB cells (10000 per well) were incubated for 24 hours in medium with different concentrations of the produced PD1-4-1BBL fusion proteins. Following incubation, IL8 concentration in the supernatant was determined by an IL8 ELISA kit according to the manufacturer's protocol. Plates were analyzed using a Plate reader (Thermo Scientific, Multiscan FC) at 450 nm, with reference at 540 nm.

Results - As shown in Figure 11, HT1080-4-1BB cells express the relevant receptor 4-1BB. All DSP105 variant proteins tested (N-terminal His-tagged DSP105_V17 (SEQ ID NO: 99), His-tagged DSP105_V22 (SEQ ID NO: 109), DSP105_V17 (SEQ ID NO: 99), and DSP105_V23 (SEQ ID NO: 13) were able to trigger the 4-1BB signaling

as determined by IL8 secretion from the HT1080-4-1BB cells, in a dose dependent manner in medium containing FBS (Figure 12).

EXAMPLE 8

5 THE EFFECT OF THE PD1-4-1BBL VARIANTS ON BLOCKING PDL1 BINDING

The PD1 part of PD1-4-1BBL is designed to block the interaction of endogenous PD1 expressed on T cells with PDL1 expressed on tumor cells. To this end, effectiveness of the produced PD1-4-1BBL variants as blockers of this interaction is evaluated.

Materials - DSP105 (HEK-NH) (SEQ ID NO: 1), DSP105 (CHO-NH) (SEQ ID NO: 1),
 10 DSP105 (CHO-NH)-V1 (SEQ ID NO: 13), DSP105 (CHO-NH)-V2 (SEQ ID NO: 15), DSP105 (CHO-NH)-V3 (SEQ ID NO: 17), N-terminal His-tagged DSP105_V17 (SEQ ID NO: 99), His-tagged DSP105_V22 (SEQ ID NO: 109), DSP105_V17 (SEQ ID NO: 99), DSP105_V18 (SEQ ID NO: 101), DSP105_V19 (SEQ ID NO: 103), DSP105_V20 (SEQ ID NO: 105) DSP105_V21 (SEQ ID NO: 107), DSP_V22 (SEQ ID NO: 111), DSP105_V23 (SEQ ID NO: 113),
 15 DSP105_V24 (SEQ ID NO: 133), DSP105_V25 (SEQ ID NO: 135), DSP105_V26 (SEQ ID NO: 137), DSP105_V27 (SEQ ID NO: 139), DSP105_V28 (SEQ ID NO: 141), DSP105_V29 (SEQ ID NO: 143), DSP105_V30 (SEQ ID NO: 145) and DSP105_V31 (SEQ ID NO: 147), produced as described in Example 2 hereinabove.

PD-1:PDL1 Inhibitor Screening ELISA Assay Pair (Acro Biosystems, cat. #EP101), including
 20 the following: recombinant human PDL1, anti hPD1 neutralizing antibody, Biotinylated hPD1.

Methods -ELISA plates are coated overnight with recombinant human PDL1. Plates are washed and incubated for 1 hour with different concentrations of the produced PD1-4-1BBL fusion proteins or the positive control anti PD1 antibody. Biotinylated PD1 is added followed by additional 1 hour incubation. Following the incubation, the plate is washed and blotted with
 25 Streptavidin-HRP and TMB substrate according to standard ELISA protocol.

Plates are analyzed using a Plate reader (Thermo Scientific, Multiscan FC) at 450 nm, with reference at 620 nm.

EXAMPLE 9

30 ACTIVATION OF PBMCs or T CELLS BY THE PD1-4-1BBL VARIANTS

The activation of a T cell requires two signals: ligation of the T-Cell Receptor (TCR) with the Major Histocompatibility Complex (MHC)/peptide complex on the Antigen Presenting Cell (APC) and cross-linking of co-stimulatory receptors on the T cell with the corresponding ligands

on the APC. 4-1BB is a T cell co-stimulatory receptor which upon ligation to 4-1BBL promotes expansion, survival, differentiation and cytokine expression of both CD8+ and CD4+ T cells.

Numerous methods are known in the art to determine activation of T cells, including but not limited to:

- 5 - Expression of activation markers on the surface of the T cells (for example: CD25, CD69, CD62L, CD137, CD107a, PD1 etc.). Expression of activation markers is tested by staining the cells with specific antibodies and flow cytometry analysis (FACS).
- Secretion of inflammatory cytokines (for example: IL2, IL6, IL8, INF gamma etc.). Secretion of inflammatory cytokine is tested by ELISA.
- 10 - Proliferation, measured by pre-staining of T cells with CFSE (carboxyfluorescein succinimidyl ester) or other cell proliferation dyes and determining deviation of cells by CFSE dilution that is determined by FACS. Proliferation is also determined using an Incucyte machine taking photos overtime and analyzing the photos with a specific software.
- Killing of a target cell e.g. cancer cell that is measured by pre - labeling the cancer cells
- 15 using Calcine-AM reagent and measuring Calcine release into the culture medium using luminescence plate reader. Killing is also determined by an Incucyte machine using labeled target cells and caspase sensitive florescent substrate.

To this end, the effect of the produced PD1-4-1BBL fusion proteins on enhancing human T cells or Peripheral Blood Mononuclear Cells (PBMCs) activation is evaluated.

- 20 *Materials* - DSP105 (HEK-NH) (SEQ ID NO: 1), DSP105 (CHO-NH) (SEQ ID NO: 1), DSP105 (CHO-NH)-V1 (SEQ ID NO: 13), DSP105 (CHO-NH)-V2 (SEQ ID NO: 15), DSP105 (CHO-NH)-V3 (SEQ ID NO: 17), N-terminal His-tagged DSP105_V17 (SEQ ID NO: 99), His-tagged DSP105_V22 (SEQ ID NO: 109), DSP105_V17 (SEQ ID NO: 97), DSP105_V18 (SEQ ID NO: 101), DSP105_V19 (SEQ ID NO: 103), DSP105_V20 (SEQ ID NO: 105), DSP105_V21 (SEQ ID NO: 107), DSP_V22 (SEQ ID NO: 111), DSP105_V23 (SEQ ID NO: 113), DSP105_V24 (SEQ ID NO: 133), DSP105_V25 (SEQ ID NO: 135), DSP105_V26 (SEQ ID NO: 137), DSP105_V27 (SEQ ID NO: 139), DSP105_V28 (SEQ ID NO: 141), DSP105_V29 (SEQ ID NO: 143), DSP105_V30 (SEQ ID NO: 145) and DSP105_V31 (SEQ ID NO: 147), produced as described in Example 2 hereinabove. Ficoll-Paque (GE Healthcare, cat# 17-1440-03), Leaf
- 25 purified Anti-human CD3 (BioLegend, cat# BLG-317315), Recombinant human IL-2 (Biolegend, cat# 589106), anti CD27, anti CD25, CD69, CD107a, CD137, PD1 antibodies, EasySep Direct Human T Cell Isolation Kit (Stemcell, cat# 19661), α CD3/CD28 dynabeads (Thermo, cat# Dy-11131D), IFN γ ELISA kit (Peprotech, cat# 900-TM27), CFSE.
- 30

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 167

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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 167

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

WHAT IS CLAIMED IS:

1. A PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL amino acid sequence, wherein said PD1 amino acid sequence:

(a) is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or is 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and does not comprise an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and/or

(b) comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and/or

wherein said 4-1BBL amino acid sequence:

(aa) is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and does not comprise an amino acid segment G198-E205 corresponding to SEQ ID NO: 3, is 170 – 182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and does not comprise an amino acid segment A1-E23 corresponding to SEQ ID NO: 3 or is 184 amino acids in length having at least 80 % identity to SEQ ID NO: 123; and/or

(bb) comprises three repeats of a 4-1BBL amino acid sequence;

and wherein said fusion protein is capable of at least one of:

(i) binding PDL1 and 4-1BB;

(ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or

(iii) co-stimulating immune cells expressing said 4-1BB.

2. A PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL amino acid sequence, wherein said PD1 amino acid sequence:

(a) is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2 and/or

(b) comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and/or

171

wherein said 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25; and wherein said fusion protein is capable of at least one of:

- (i) binding PDL1 and 4-1BB;
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
- (iii) co-stimulating immune cells expressing said 4-1BB.

3. A PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL amino acid sequence, wherein said PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and/or wherein said 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25; and wherein said fusion protein is capable of at least one of:

- (i) binding PDL1 and 4-1BB;
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
- (iii) co-stimulating immune cells expressing said 4-1BB.

4. A PD1-4-1BBL fusion protein comprising a (GGGGS)_n(n=1-4) linker between said PD1 and said 4-1BBL, wherein said fusion protein is capable of at least one of:

- (i) binding PDL1 and 4-1BB;
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
- (iii) co-stimulating immune cells expressing said 4-1BB.

5. An isolated polypeptide comprising a PD1 amino acid sequence, wherein said PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or is 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and does not comprise an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and optionally comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and wherein said polypeptide is capable of binding PDL1.

172

6. An isolated polypeptide comprising a PD1 amino acid sequence, wherein said PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and optionally comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and wherein said polypeptide is capable of binding PDL1.

7. An isolated polypeptide comprising a PD1 amino acid sequence, wherein said PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and wherein said polypeptide is capable of binding PDL1.

8. An isolated polypeptide comprising a 4-1BBL amino acid sequence, wherein said 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and does not comprise an amino acid segment G198-E205 corresponding to SEQ ID NO: 3, or is 170 – 182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and does not comprise an amino acid segment A1-E23 corresponding to SEQ ID NO: 3; and optionally comprises three repeats of said 4-1BBL amino acid sequence; and wherein said polypeptide is capable of at least one of:

- (i) binding 4-1BB,
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
- (iii) co-stimulating immune cells expressing said 4-1BB.

9. An isolated polypeptide comprising a 4-1BBL amino acid sequence, wherein said 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25; and wherein said polypeptide is capable of at least one of:

- (i) binding 4-1BB,
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
- (iii) co-stimulating immune cells expressing said 4-1BB.

10. The PD1-4-1BBL fusion protein of any one of claims 1-4 or the isolated polypeptide of any one of claims 5-7, wherein said PD1 amino acid sequence is no more than 140 amino acids long.

11. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-7 and 10, wherein said PD1 amino acid sequence is at least 126 amino acids long.

12. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1, 5 and 10-11, wherein said PD1 amino acid sequence comprises said SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 or 115.

13. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-2, 4-6 and 10-11, wherein said PD1 amino acid sequence comprises SEQ ID NO: 75, 83, 87, 91 or 95.

14. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1, 5 and 10-11, wherein said PD1 amino acid sequence consists of said SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 or 115.

15. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-2, 4-6 and 10-11, wherein said PD1 amino acid sequence consists of SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95.

16. The PD1-4-1BBL fusion protein of or the isolated polypeptide any one of claims 1-2, 4 and 8-15, wherein said 4-1BBL amino acid sequence does not comprise any of amino acid residues A1 – V6 or A1 – G14 corresponding to SEQ ID NO: 3.

17. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-2, 4 and 8-16, wherein said 4-1BBL amino acid sequence comprises said SEQ ID NO: 22, 23, 24 or 25.

18. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-4 and 8-16, wherein said 4-1BBL amino acid sequence consists of said SEQ ID NO: 22, 23, 24 or 25.

19. The PD1-4-1BBL fusion protein of any one of claims 1-3 and 10-18, wherein the PD1-4-1BBL fusion protein comprises a linker between said PD1 and said 4-1BBL.

20. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1, 8, and 10-18, comprising a linker between each of said three repeats of said 4-1BBL amino acid sequence.

21. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 19-20, wherein the linker is a single amino acid linker.

22. The PD1-4-1BBL fusion protein or the isolated polypeptide of claim 21, wherein the linker is glycine.

23. The PD1-4-1BBL fusion protein of claim 19, wherein the linker is an Fc domain of an antibody or a fragment thereof.

24. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 19-20, wherein the linker is a (GGGGS)_n(n=1-4) linker.

25. The PD1-4-1BBL fusion protein of any one of claims 1-4 and 10-24, wherein production yield of said fusion protein is at least 1.5 fold higher than the production yield of SEQ ID NO: 5 under the same production conditions, said production conditions comprise expression in a mammalian cell and culturing at 32 – 37 °C, 5 – 10 % CO₂ for 5 - 13 days.

26. The PD1-4-1BBL fusion protein of any one of claims 1-4 and 10-24, wherein the amount of aggregates of said fusion protein is at least 20 % lower than the amount of aggregates of SEQ ID NO: 5 under the same production conditions, said aggregates are of at least 300 kDa in molecular weight and said production conditions comprise expression in a mammalian cell and culturing at 32 – 37 °C, 5 – 10 % CO₂ for 5 - 13 days.

27. The PD1-4-1BBL fusion protein of any one of claims 1, 19 and 25-26, wherein said PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147.

28. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-8 and 10-27, wherein said at least 80 % identity comprises at least 90 % identity.

29. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-8 and 10-27, wherein said at least 80 % identity comprises at least 95 % identity.

30. The PD1-4-1BBL fusion protein of any one of claims 1, 10-19 and 25-29, wherein said PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147.

31. The PD1-4-1BBL fusion protein of any one of claims 1, 10-19 and 25-29, wherein said PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107, 111, 113, 133, 135, 137, 139, 141, 143 and 147.

32. A polynucleotide encoding the PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-31.

33. A nucleic acid construct comprising the polynucleotide of claim 32, and a regulatory element for directing expression of said polynucleotide in a host cell.

34. A host cell comprising the PD1-4-1BBL fusion protein or the polypeptide of any one of claims 1-31 or the polynucleotide or the nucleic acid construct of any one of claims 32-33.

35. A method of producing a PD1-4-1BBL fusion protein or a polypeptide, the method comprising expressing in a host cell the polynucleotide or the nucleic acid construct of any one of claims 32-33.

36. The method of claim 35, comprising isolating the fusion protein or the polypeptide.

37. A method of treating a disease that can benefit from activating immune cells comprising administering to a subject in need thereof the PD1-4-1BBL fusion protein or the

isolated polypeptide of any one of claims 1-31, the polynucleotide or the nucleic acid construct of any one of claims 32-33 or the host cell of claim 34.

38. The method of claim 37, further comprising administering to said subject a therapeutic agent for treating said disease.

39. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-31, the polynucleotide or the nucleic acid construct of any one of claims 32-33 or the host cell of claim 34, for use in treating a disease that can benefit from activating immune cells.

40. The composition for use of claim 39, further comprising a therapeutic agent for treating said disease.

41. An article of manufacture comprising a packaging material packaging a therapeutic agent for treating a disease that can benefit from activating immune cells; and the PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-31, the polynucleotide or the nucleic acid construct of any one of claims 32-33 or the host cell of claim 34.

42. The method, the composition for use or the article of manufacture of any one of claims 37-41, wherein cells of said disease express PDL1.

43. The method, the composition for use or the article of manufacture of any one of claims 37-42, wherein said disease comprises a hyper-proliferative disease.

44. The method, the composition for use or the article of manufacture of claim 43, wherein said hyper-proliferative disease comprises cancer.

45. The method, the composition for use or the article of manufacture of claim 44, wherein said cancer is selected from the group consisting of lymphoma, leukemia and carcinoma.

46. The method, the composition for use or the article of manufacture of any one of claims 37-42, wherein said disease comprises a disease associated with immune suppression or medication induced immunosuppression or an infection.

47. A method of activating immune cells, the method comprising in-vitro activating immune cells in the presence of the PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-31, the polynucleotide or the nucleic acid construct of any one of claims 32-33 or the host cell of claim 34.

48. The method of claim 47, wherein said activating is in the presence of cells expressing PDL1 or exogenous PDL1.

49. The method of any one of claims 47-48, wherein said activating is in the presence of an anti-cancer agent.

50. The method, the composition for use or article of manufacture of any one of claims 38, 40 – 46 and 49, wherein said therapeutic agent for treating said disease or said anti-cancer agent comprises an antibody.

FIG. 1

MEAPAQLLFLLLLLWLPDTTG**HHHHHH**PGWFLDSPDRPWNPTFS
 PALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLA
 PEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSTYLCGAI
 SLAPKAQIKESLRAELRVTERRAEVPTAHPSPPSPRPAGQFQTLV
 GACPWAVSGARASPGSAASPRRLREGPELSPDDPAGLLDLRQGMF
 AQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVA
 KAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALA
 LTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARH
 AWQLTQGATVLGLFRVTPEIPAGLPSPRSE

FIG. 2A

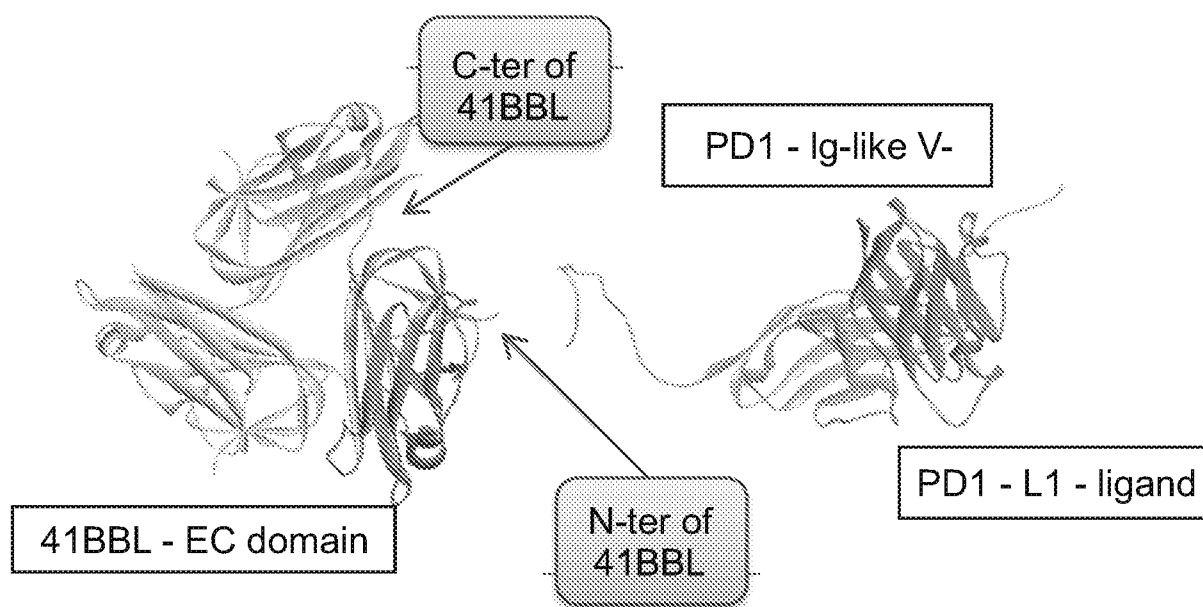


FIG. 2B

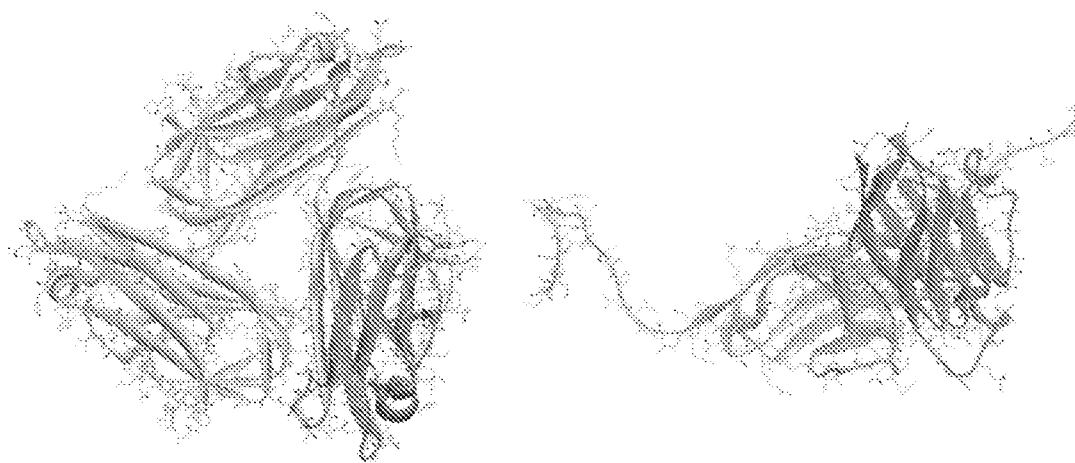


FIG. 2C

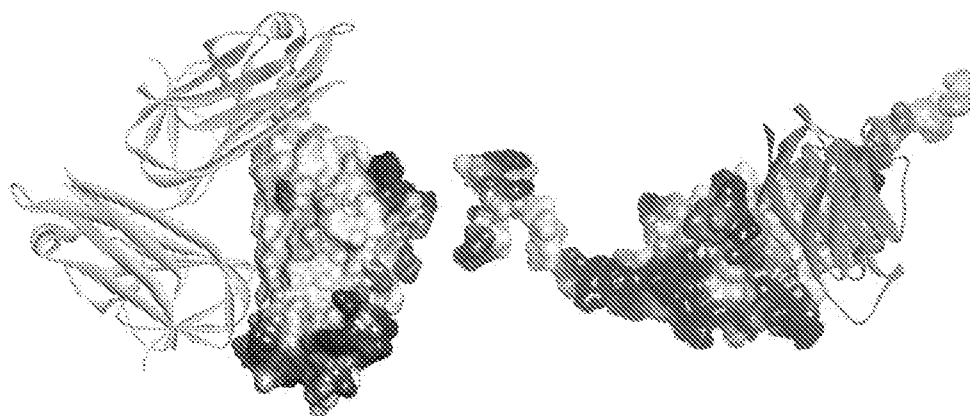


FIG. 2D

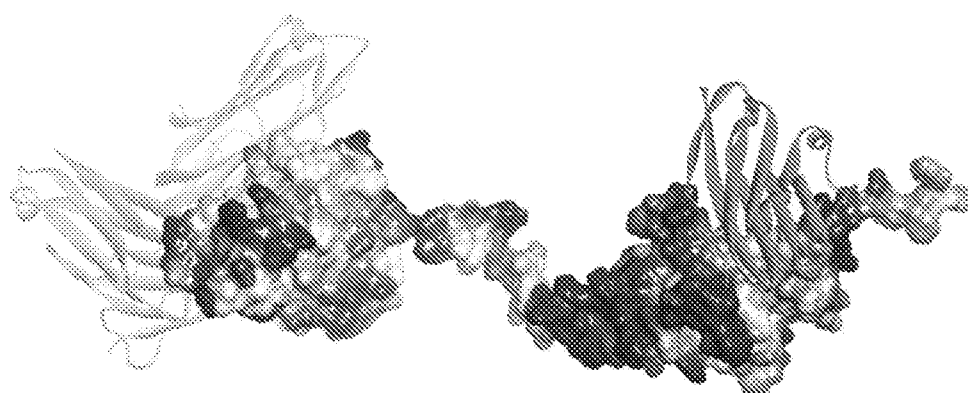


FIG. 3

PGWFELDSPDRPWNEPTTSPALLVTEGDNATFTCSFSNISESEVLNNYRMSPEKLDKLAEPED
 KSCFQDELRVTCLNEDTMSVVAQNDGGTYLGGATSLAFACTKSLRAELRYTERRAE
 VPTAHPSPSERPAGQFQTLVGACPWAVSGARASPGSAASPRLREGPELSPDOPAGLLDLRQGMFA
 QLVAQNVLLIDGFLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRVVGEGS
 GSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARAR
 HAWQLTQGATVLGLFRVTPEIPAGLPSRSE

FIG. 4

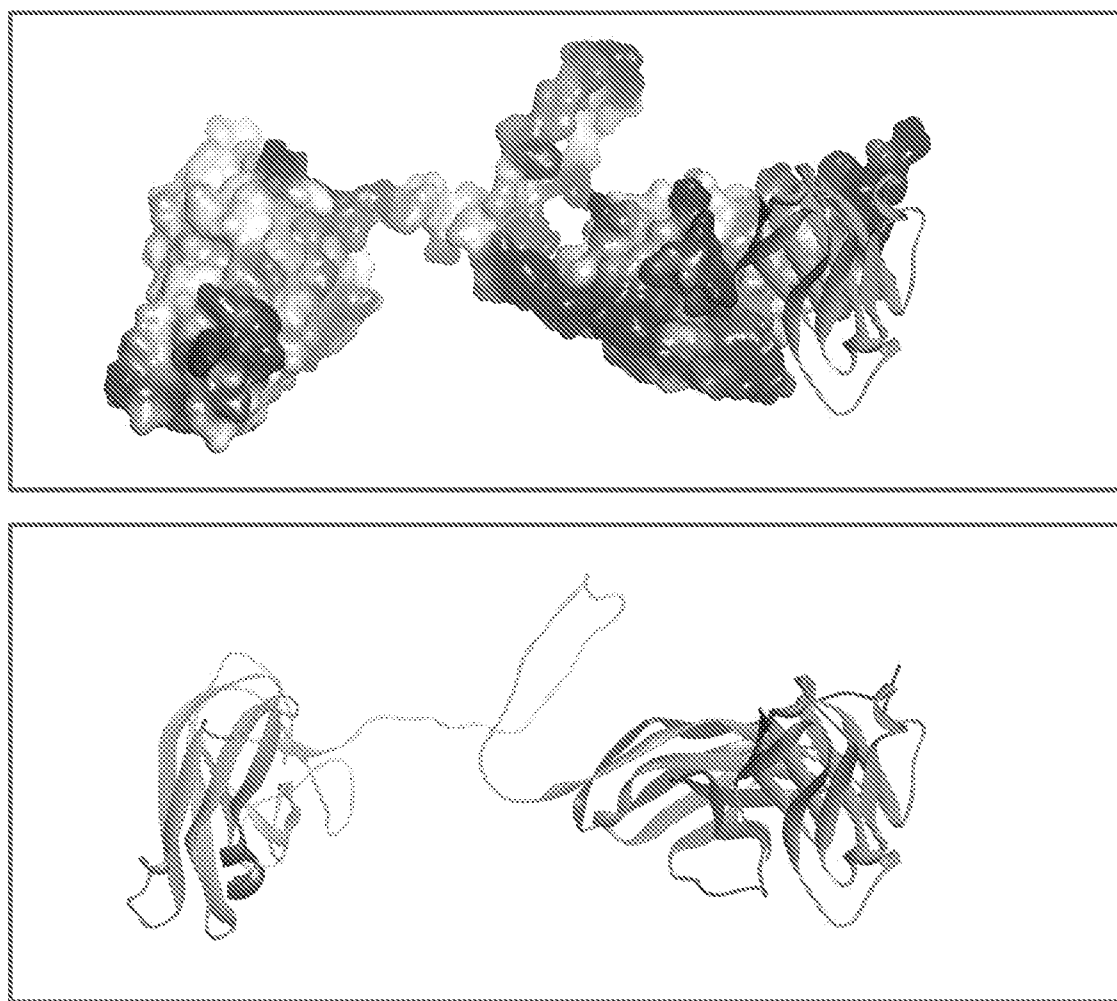


FIG. 5

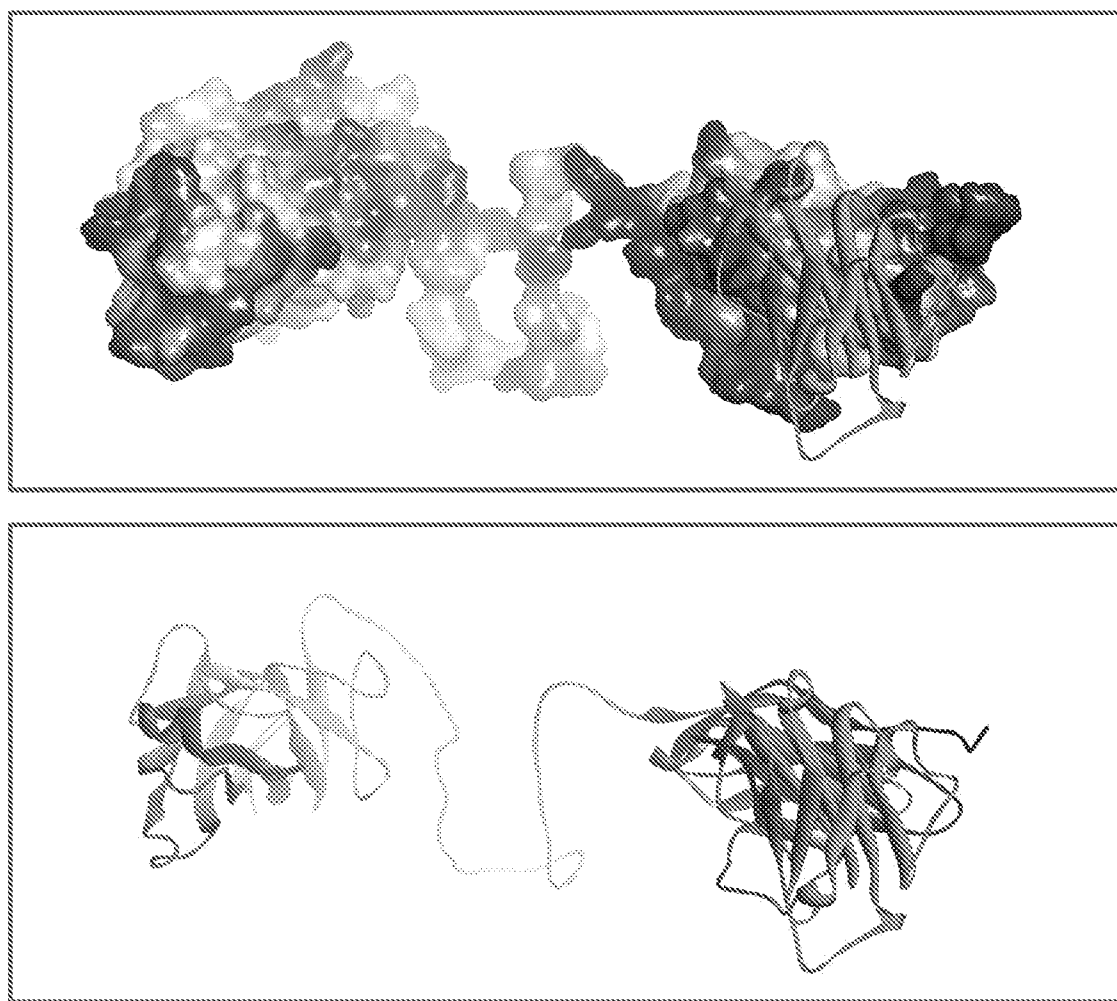


FIG. 6

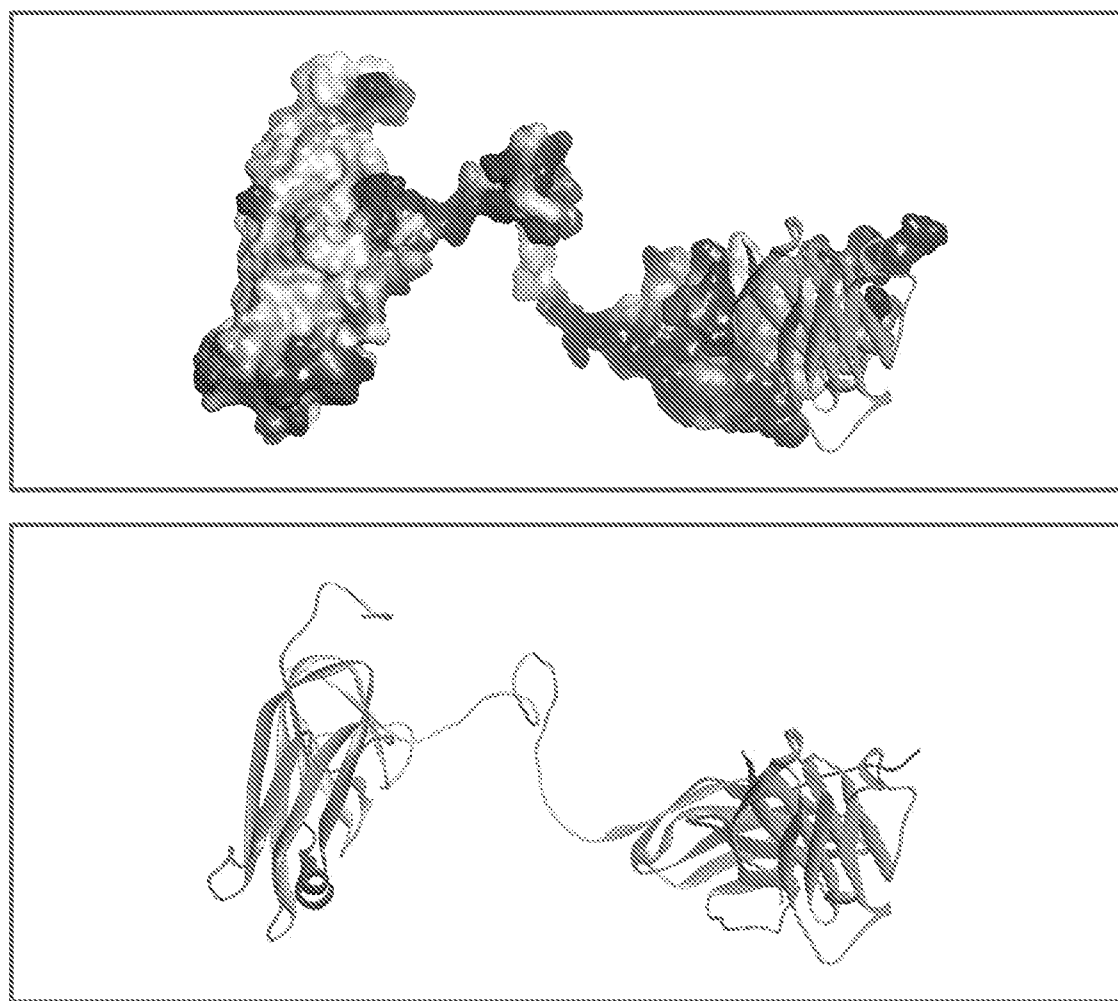


FIG. 7

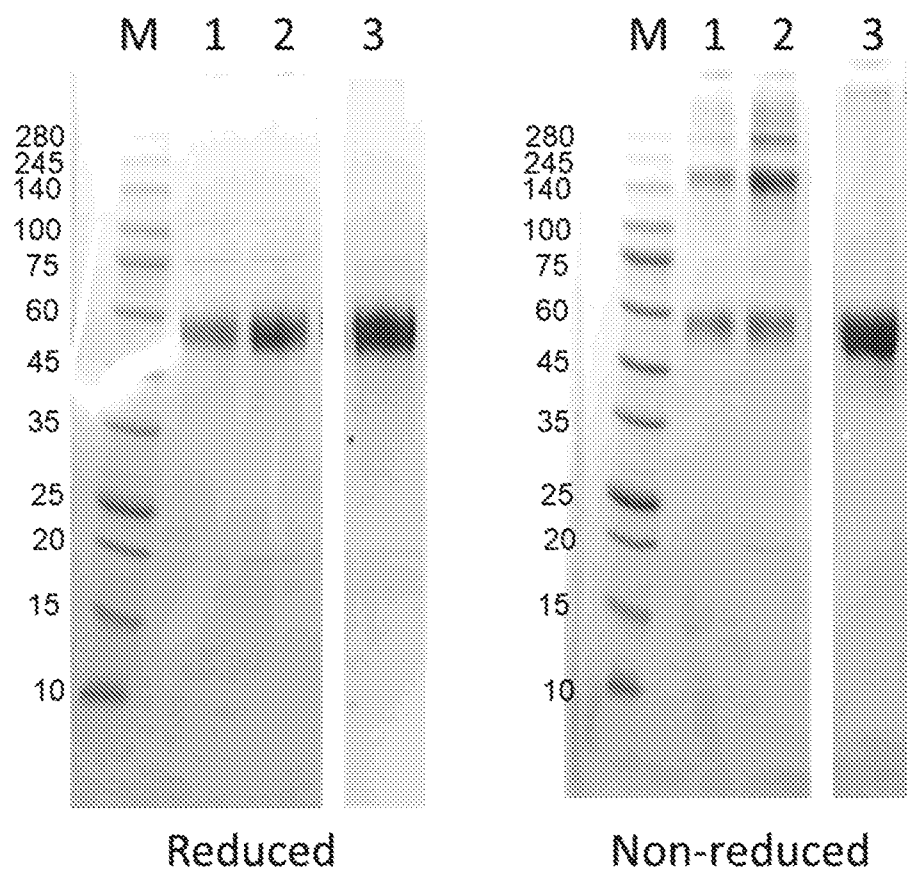


FIG. 8A

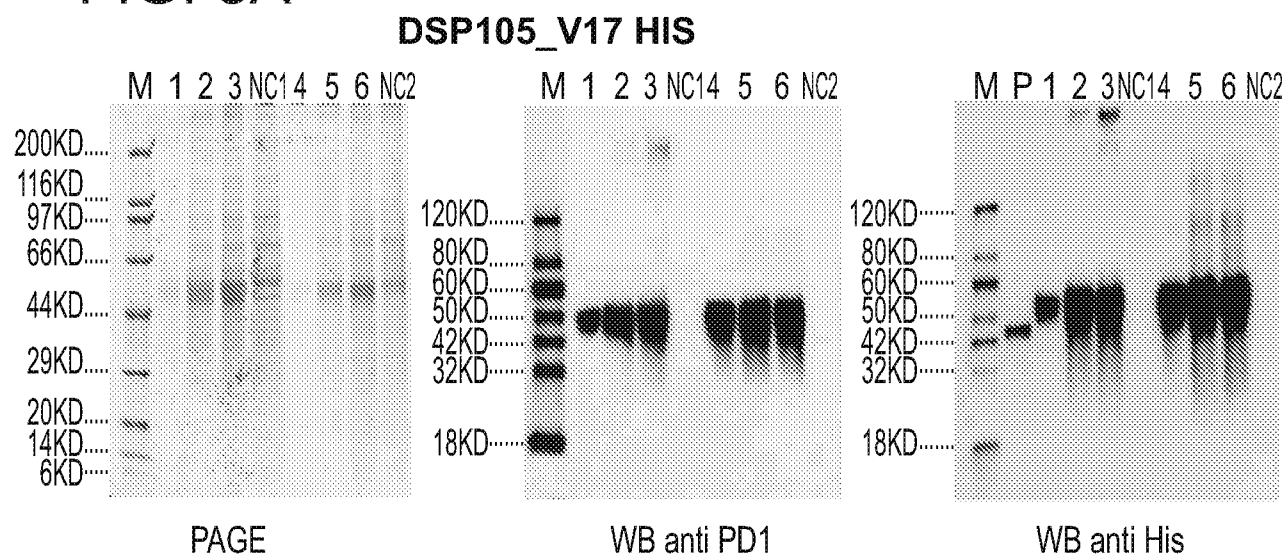


FIG. 8B

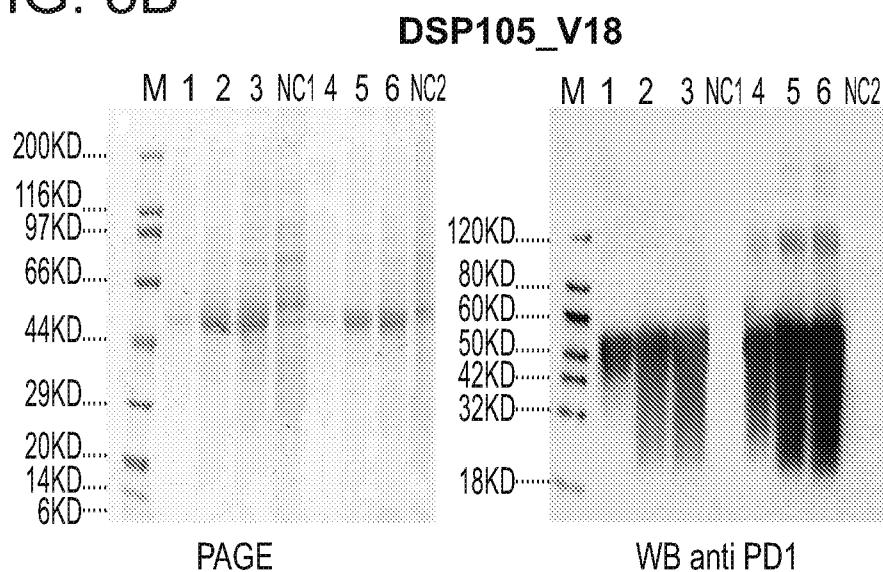


FIG. 8C

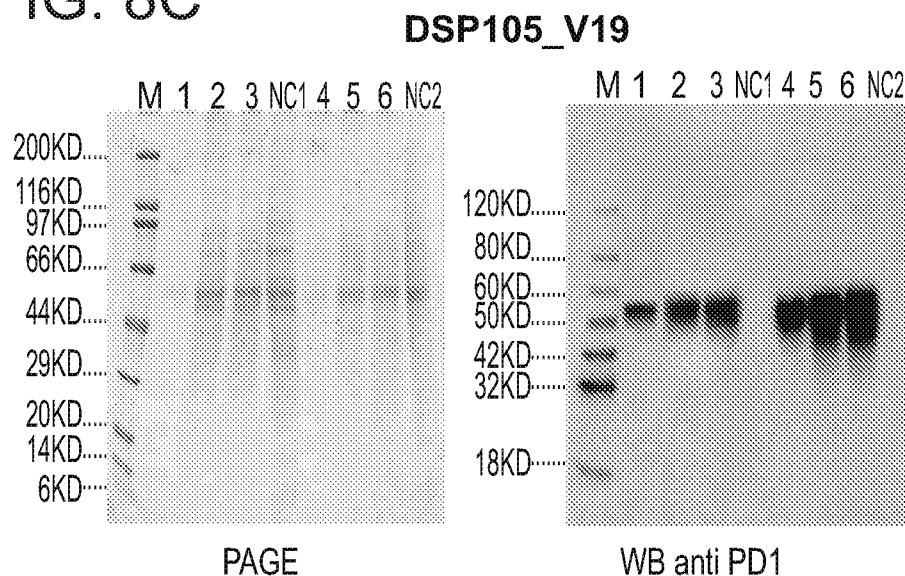


FIG. 8D

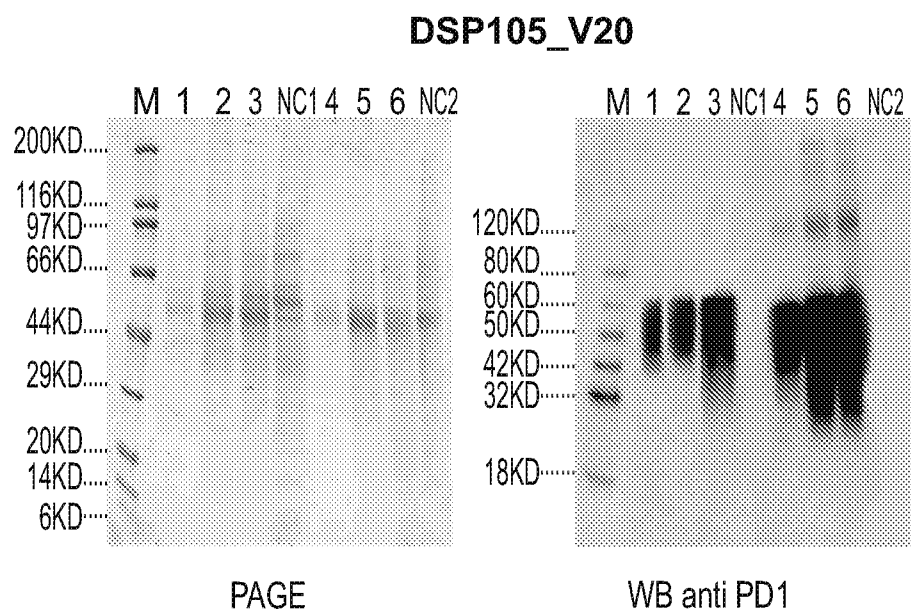


FIG. 8E

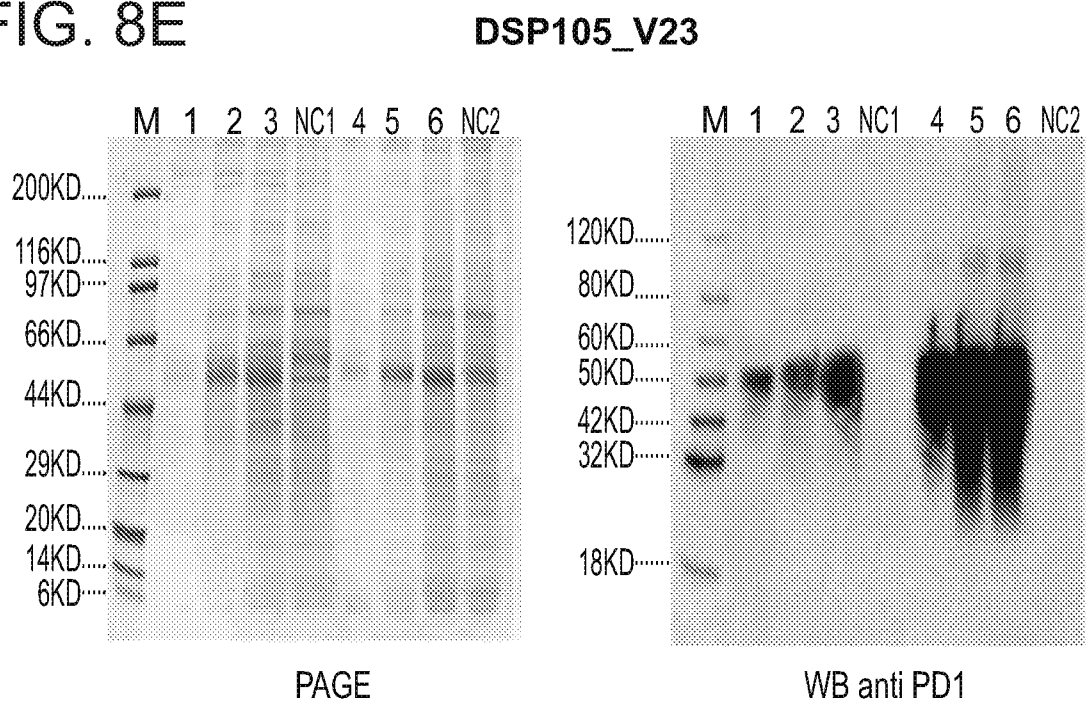


FIG. 9A

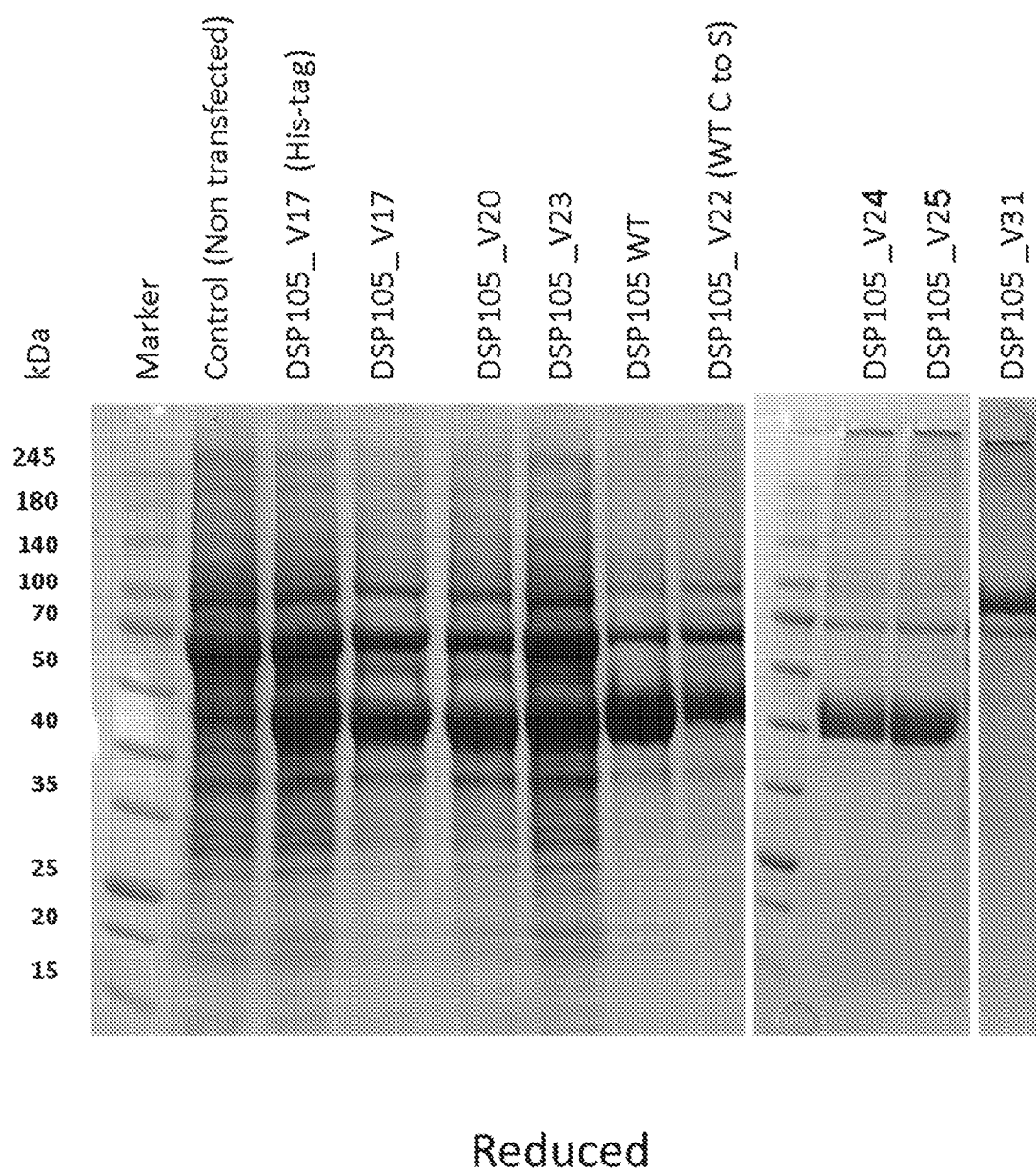
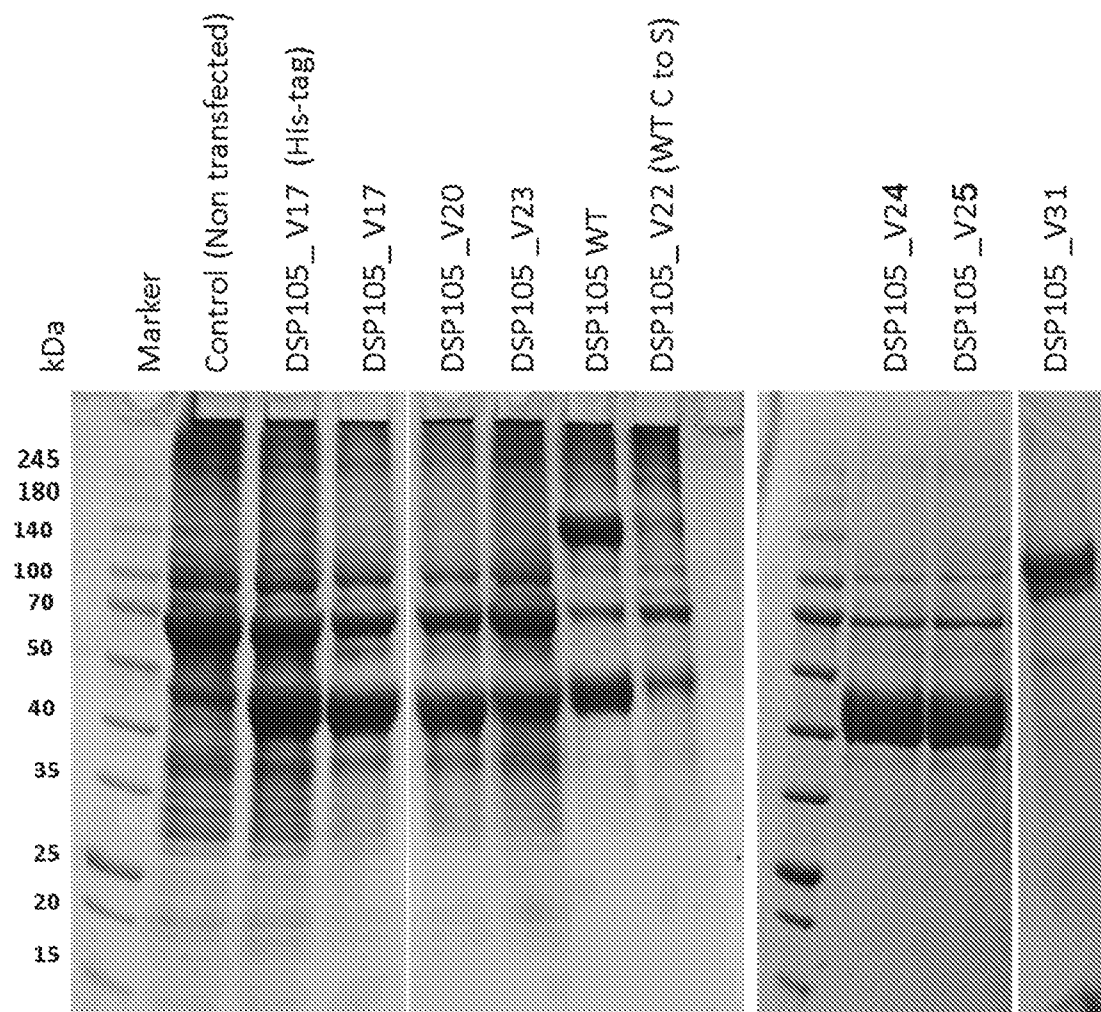


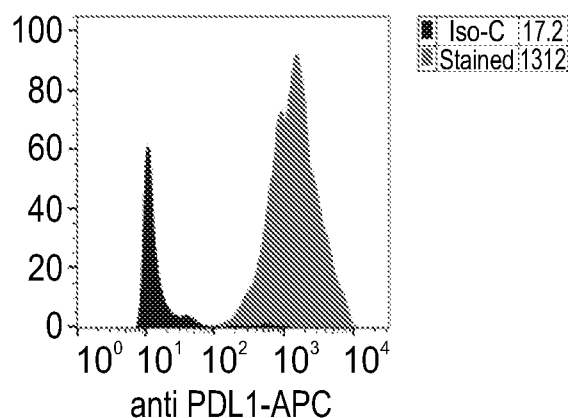
FIG. 9B



Non-Reduced

FIG. 10A

PDL1 expression on DLD1-PDL1 cells



41BB expression on HT1080-41BB cells

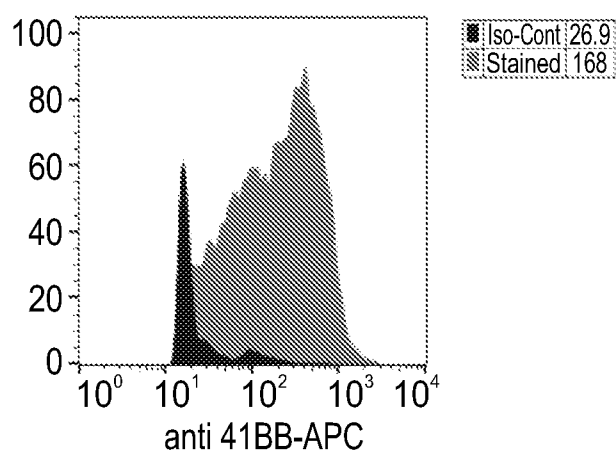


FIG. 10B

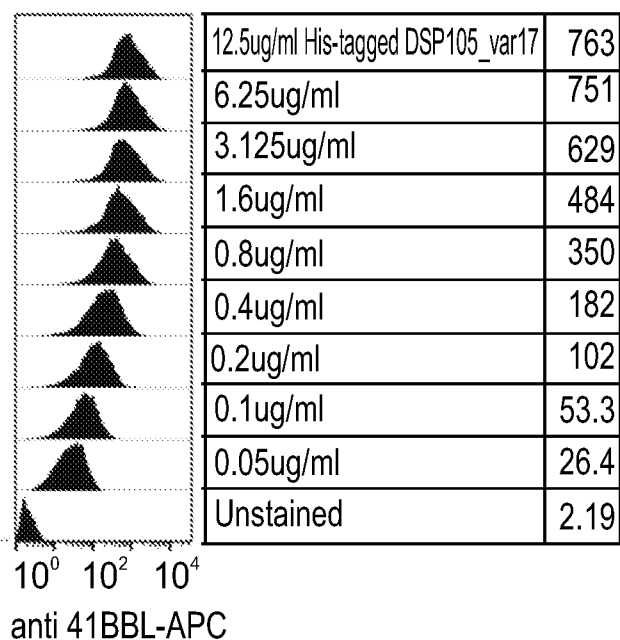
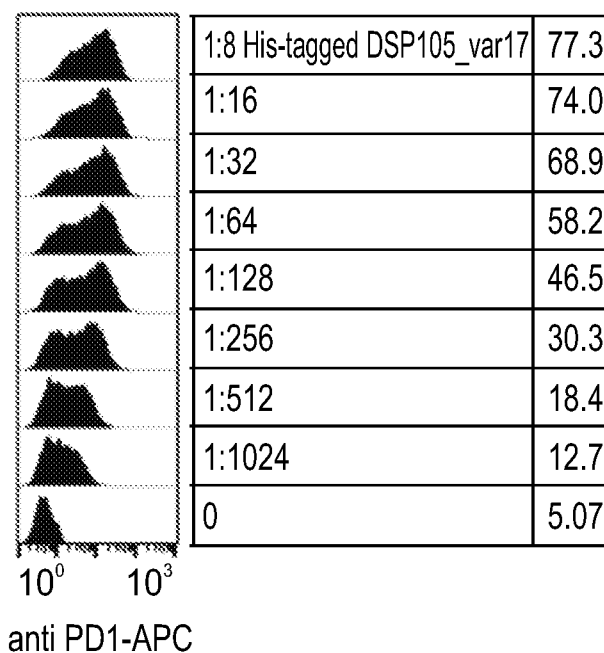
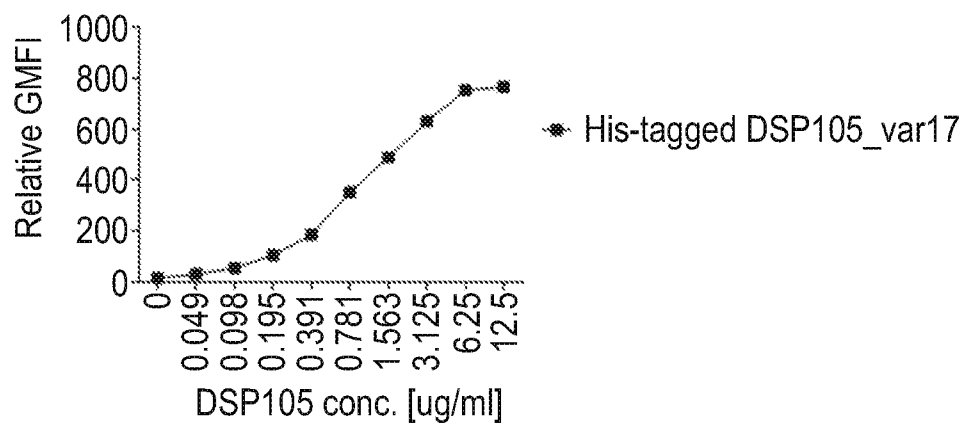
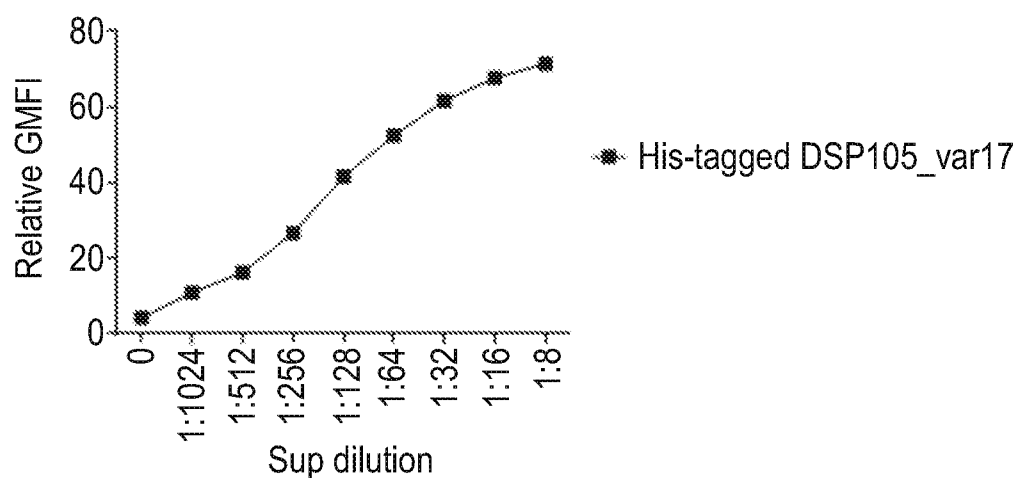
Binding of His-tagged DSP105_var17
to DLD1-PDL1 cellsBinding of His-tagged DSP105_var17
to HT1080-41BB cells

FIG. 10C

Graphic presentation of His-tagged DSP105_var17
binding to DLD1-PDL1 cells



Graphic presentation of His-tagged DSP105_var17
binding to HT1080-41BB cells

**FIG. 11**

41BB expression on HT1080-41BB cells

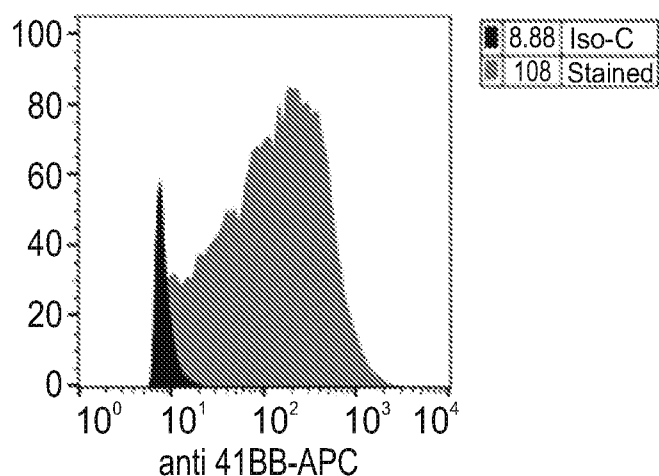


FIG. 12

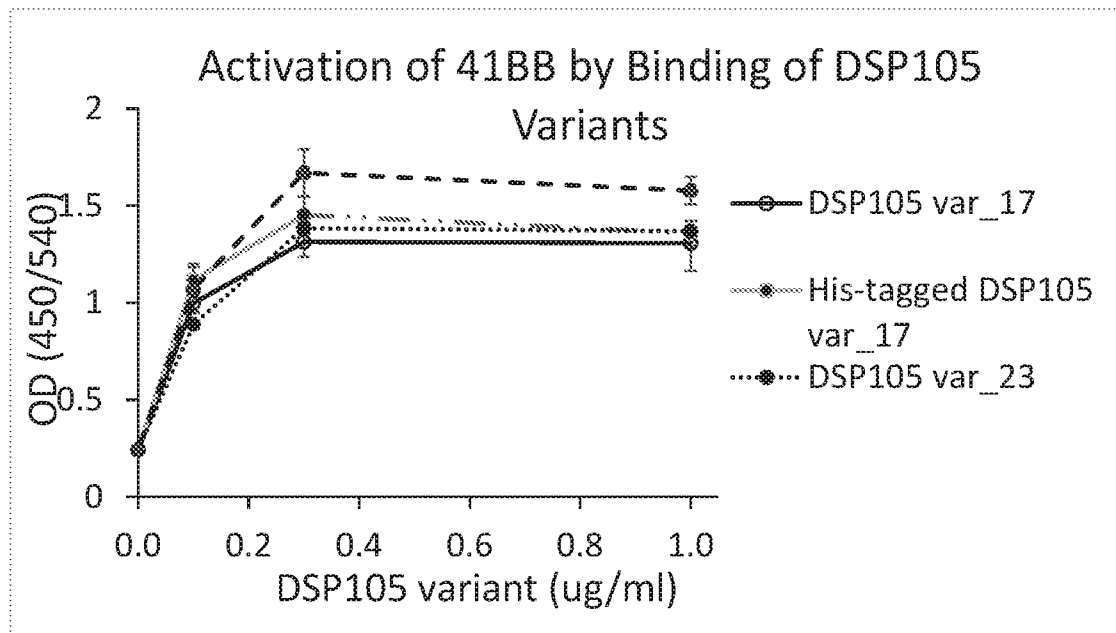


FIG. 13A

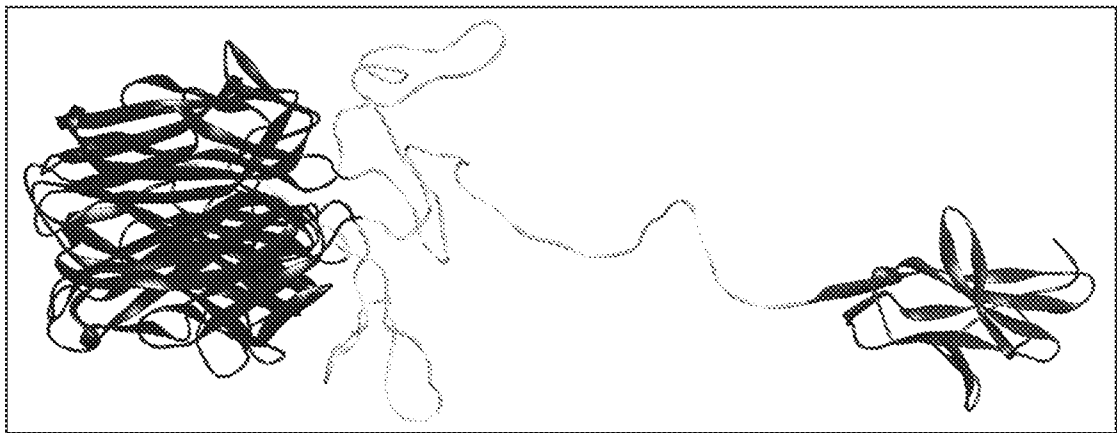


FIG. 13B

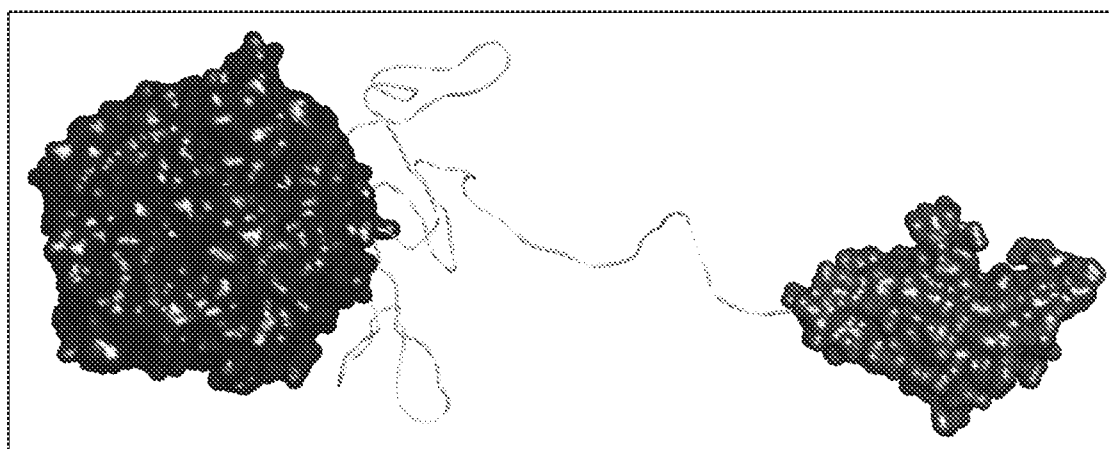


FIG. 13C

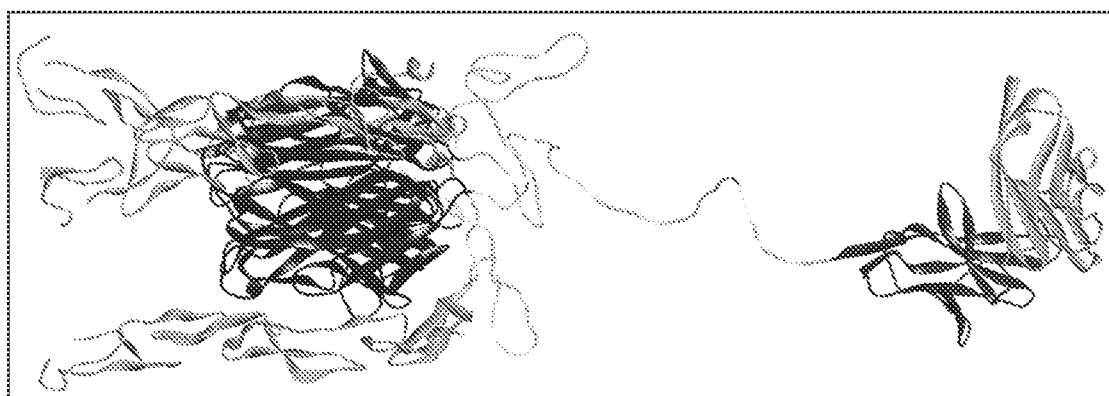


FIG. 13D

