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(54) Title: ENGINEERED T-CELL MODULATING MOLECULES AND METHODS OF USING SAME

(57) Abstract: ABSTRACT Provided herein are engineered nucleic acids, engineered mRNAs, engineered polypeptides, and engineered pentamerized polypeptides, human and murine, each of which include sequences of VISTA, ICOS-L, PD-L1 or B7-H4 extracellular domains operably linked to the pentamerization domain of COMP. A soluble form of the pentamerized polypeptides has T-cell inhibitory activity, in the case of VISTA, PD-L1 or B7H4, or T-cell stimulatory activity, in the case of ICOS-L. Methods of using same for treatment of a subject in need of T-cell modulating activity are provided.

ENGINEERED T-CELL MODULATING MOLECULES AND METHODS OF USING SAME**CROSS REFERENCE TO PRIOR APPLICATIONS**

[0001] This application claims priority under the Paris Convention to U.S. Provisional Patent Application 62/478,198, filed March 29, 2017, and U.S. Provisional Patent Application 62/590,848, filed November 27, 2017, each of which are incorporated herein by reference as if set forth in their entirety.

FIELD OF THE DISCLOSURE

[0002] This present disclosure relates generally to engineered T-cell modulating molecules. In particular, the present disclosure is directed to engineered V-domain Immunoglobulin suppressor of T-cell activation (VISTA), programmed death-ligand 1 (PD-L1) and B7 homolog 4 (B7-H4) molecules that inhibit T-cells and engineered inducible costimulatory ligand (ICOS-L) molecules that stimulate T-cells.

BACKGROUND OF THE DISCLOSURE

[0003] T-cell activity is regulated by co-stimulatory and co-inhibitory signals generated by the binding of immune checkpoint (IC) cell surface molecules present on T-cells and antigen presenting cells (APC)/cancer cells. These signals facilitate protection against invading pathogens and/or malignant cells, while maintaining self-tolerance. T-cell responses are either upregulated by co-stimulatory checkpoint pairs exemplified by CD28:CD80/CD86, ICOS:ICOS-L, OX-40:OX-40L and 4-1BB:4-1BBL and/or downregulated by co-inhibitory checkpoint molecules which include CTLA-4:CD80/CD86, PD-1:PD-L1 (References 1-4). Some T cell co-inhibitory IC pairs have not yet been fully characterized including the receptors recognized by VISTA and B7-H4. To date, several negative checkpoint receptors that function to suppress T-cell activity have been identified, including PD-1 and CTLA-4. Antibody-mediated blockade of these pathways has been shown to promote anti-tumor immune responses (References 1-3) while ligands which activate these immunoinhibitory pathways may suppress uncontrolled immune responses linked to autoimmune and/or inflammatory disorders (References 4-6). In contrast, activation of co-stimulatory pathways in T-cells such as ICOS:ICOS-L and OX40:OX40L can lead to T-cell activation, proliferation and cytokine production; outcomes which can promote anti-tumor immune responses.

[0004] Many of the known immune checkpoint pairs are members of the B7-CD28 family of surface proteins defined in part by their Ig-V/Ig-C containing extracellular domains. These

pairs include ICOS:ICOS-L, CTLA-4:CD80/CD86, PD-1:PD-L1, as well as VISTA and B7-H4 (Reference 7).

[0005] V-domain Immunoglobulin suppressor of T-cell activation ("VISTA", which may also be referred to as PD-1H, DD1 α , SISP1, Dies1, c10Orf54, and/or Gi24) is a checkpoint ligand that is expressed primarily on CD11b^{high} myeloid cells and which negatively regulates T-cell responses upon binding to a putative cell surface receptor (VISTA-receptor) (References 8-9). VISTA is also expressed on naïve CD4⁺ and CD8⁺ T-cells, where it is postulated to negatively regulate T-cell responses, suggesting a dual-role of VISTA as both a checkpoint ligand and receptor (Reference 10). Structurally, VISTA shares significant homology with PD-1 and PD-L1, having an N-terminal IgV domain followed by a single membrane spanning domain and cytoplasmic tail. Similar to the PD-1:PD-L1 pathway, blockade of VISTA using monoclonal antibodies has been demonstrated to provoke anti-tumor immune responses in mouse models, suggesting a role for VISTA:VISTA-receptor signalling in the promotion of tumor immune evasion (References 11-12). VISTA may also play a role in regulating autoimmune disease progression. For example, VISTA-deficient (VISTA^{-/-}) mice bred on a lupus-prone background developed accelerated and severe systemic lupus erythematosus (SLE) (Reference 13). VISTA^{-/-} 2D2 T-cell receptor transgenic mice exhibited increased levels of peripheral encephalitogenic T-cells and developed an exacerbated form of experimental autoimmune encephalomyelitis (EAE) (Reference 14). VISTA^{-/-} mice bred on a C57Bl/6 background displayed a mild pro-inflammatory phenotype, exemplified by an increase in dendritic cells and a rise in T-cell activation markers, but were not reported to develop inflammatory disorders (Reference 14). In slight contrast, another study described a more severe phenotype, where VISTA^{-/-} C57Bl/6 mice developed glomerulonephritis at 10 months of age (Reference 15). Together, these studies suggest that promoting VISTA-mediated immunosuppression may be useful for treatment of autoimmune and/or inflammatory diseases.

[0006] Agonistic anti-VISTA antibodies have been reported (Reference 16) and a dimeric version of VISTA (VISTA.Fc) has been reported to suppress T-cell activation in-vitro when VISTA.Fc is immobilized on a solid surface (References 8, 11).

[0007] The inhibitory co-stimulatory molecule known as programmed death-1 (PD-1) is expressed on activated T cells, B cells, monocytes, and macrophages and binds to PD-L1 (on hematopoietic and non-hematopoietic cells) and PD-L2 (on DCs and macrophages) (References 6, 17-20). PD-L1 binding to PD-1 on lymphocytes sends an inhibitory signal to T cells that blocks TCR signaling, T- and B-cell proliferation, cytokine production and CD8⁺ T cell cytotoxicity (References 4, 17). PDL-2 is a second ligand for PD-1 and inhibits T-cell

activation (Reference 20). The PD-1 agonist PD-L1.Fc has been shown to improve disease outcome in two CIA mouse models (References 5, 21).

[0008] B7-H4 is another B7 family member that is an IgV domain-containing inhibitory ligand. Its receptor first presumed to be BTLA-4 still remains unknown (Reference 22). B7-H4.Fc has been shown to dampen immune responses in vivo, exemplified by its ability to reduce ConA-induced hepatic injury in mice (Reference 23) and to limit the progression of CIA in mice (Reference 24).

[0009] The Inducible T-cell Costimulator ((ICOS), which may also be referred to as CD278, H4 or AILIM) is a receptor in the CD28 family of B7-binding proteins (References 25-27) which is inducibly expressed on activated T cells (References 25, 28, 29). Upon binding to its ligand ICOS-L (B7-H2) expressed on APCs (References 30, 31), T-cells are co-stimulated by ICOS to enhance Th1 and Th2 functions reflected by the production of effector cytokines (IL-4, IL-5, IL-10, IL-21, IFN γ , TNF α) (References 32-34).

[0010] Pre-clinical tumor studies have shown that mice implanted with tumor cells expressing ICOS-L (to agonize ICOS signalling) have reduced tumor growth and improved survival in the context of anti-CTLA-4 therapy (Reference 35). As well, a clinical trial of patients treated with anti-CTLA-4 agents have shown that the presence of ICOS-hi T cells correlates with an increased treatment response to these immune checkpoint inhibitors (References 36, 37). These results suggest the use of an ICOS agonist as a strategy to enhance anti-tumor immune response. PCT Application No. WO2016US23524 describes agonistic monoclonal antibodies that target ICOS. These antibodies are purported to both stimulate immune cells to kill tumor cells, and to limit the number of Treg linked to the suppression of antitumor immunity.

[0011] There is currently a need for improved compounds and methods for regulating or modulating T-cell activity, inducing immunosuppression and/or improving anti-tumor immune responses.

SUMMARY OF THE DISCLOSURE

[0012] Provided herein are engineered nucleic acids, engineered mRNAs, engineered polypeptides, and engineered pentamerized polypeptides, human and murine, each of which includes a sequence of a VISTA, B7-H4, PD-L1 or ICOS-L extracellular domain operably linked to the pentamerization domain of COMP. A soluble form of the pentamerized polypeptides has T-cell modulating activity in vitro and in vivo. Methods of using same for treatment of a subject in need of T-cell modulating activity are also provided.

[0013] In an aspect, a recombinant nucleic acid is provided. The recombinant nucleic acid comprises: a nucleic acid having substantial similarity to a nucleic acid encoding an extracellular IgV-containing domain of a V-domain Ig Suppressor of T cell Activation (VISTA) having a sequence of SEQ ID NO: 1 or 2; and a nucleic acid having substantial similarity to a nucleic acid encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 3 or 4, the nucleic acid encoding the extracellular IgV-domain containing VISTA polypeptide being operably linked to the nucleic acid encoding the pentamerization domain of COMP.

[0014] In an embodiment, the recombinant nucleic acid comprises SEQ ID NO: 1 operably linked to SEQ ID NO: 3. In an embodiment, the recombinant nucleic acid comprises SEQ ID NO: 2 operably linked to SEQ ID NO: 4.

[0015] In another aspect, a recombinant nucleic acid is provided. The recombinant nucleic acid comprises: a nucleic acid having substantial similarity to a nucleic acid encoding an extracellular domain of B7-H4 having a sequence of SEQ ID NO: 26; and a nucleic acid having substantial similarity to a nucleic acid encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 3, the nucleic acid encoding the extracellular domain of B7-H4 polypeptide being operably linked to the nucleic acid encoding the pentamerization domain of COMP.

[0016] In another aspect, a recombinant nucleic acid is provided. The recombinant nucleic acid comprises: a nucleic acid having substantial similarity to a nucleic acid encoding an extracellular domain of PD-L1 having a sequence of SEQ ID NO: 37; and a nucleic acid having substantial similarity to a nucleic acid encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 3, the nucleic acid encoding the extracellular domain of PD-L1 polypeptide being operably linked to the nucleic acid encoding the pentamerization domain of COMP.

[0017] In another aspect, a recombinant nucleic acid is provided. The recombinant nucleic acid comprises: a nucleic acid having substantial similarity to a nucleic acid encoding an extracellular domain of ICOS-L having a sequence of SEQ ID NO: 48; and a nucleic acid having substantial similarity to a nucleic acid encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 3, the nucleic acid encoding the extracellular domain of ICOS-L polypeptide being operably linked to the nucleic acid encoding the pentamerization domain of COMP.

[0018] In an embodiment, an expression vector comprising the recombinant nucleic acid of the disclosure is provided. In an embodiment, the expression vector further comprises at

least one control sequence. In an embodiment, a host cell comprising the expression vector is provided.

[0019] In an aspect, a recombinant messenger ribonucleic acid (mRNA) is provided. The mRNA comprises: an mRNA having substantial similarity to an mRNA encoding an extracellular domain of a V-domain Ig Suppressor of T cell Activation (VISTA) having a sequence of SEQ ID NO: 5 or 6; and an mRNA having substantial similarity to an mRNA encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 7 or 8, the mRNA encoding the extracellular domain of VISTA mRNA being operably linked to the mRNA encoding the pentamerization domain of COMP.

[0020] In an embodiment, the recombinant mRNA comprises SEQ ID NO: 5 operably linked to SEQ ID NO: 7. In an embodiment, the recombinant mRNA comprises SEQ ID NO: 6 operably linked to SEQ ID NO: 8.

[0021] In an aspect, a recombinant messenger ribonucleic acid (mRNA) is provided. The mRNA comprises: an mRNA having substantial similarity to an mRNA encoding an extracellular domain of B7-H4 having a sequence of SEQ ID NO: 27; and an mRNA having substantial similarity to an mRNA encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 7, the mRNA encoding the extracellular domain of B7-H4 mRNA being operably linked to the mRNA encoding the pentamerization domain of COMP.

[0022] In an aspect, a recombinant messenger ribonucleic acid (mRNA) is provided. The mRNA comprises: an mRNA having substantial similarity to an mRNA encoding an extracellular domain of PD-L1 having a sequence of SEQ ID NO: 62; and an mRNA having substantial similarity to an mRNA encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 7, the mRNA encoding the extracellular domain of PD-L1 mRNA being operably linked to the mRNA encoding the pentamerization domain of COMP.

[0023] In an aspect, a recombinant messenger ribonucleic acid (mRNA) is provided. The mRNA comprises: an mRNA having substantial similarity to an mRNA encoding an extracellular domain of ICOS-L having a sequence of SEQ ID NO: 61; and an mRNA having substantial similarity to an mRNA encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 7, the mRNA encoding the extracellular domain of ICOS-L mRNA being operably linked to the mRNA encoding the pentamerization domain of COMP.

[0024] In an aspect, a recombinant polypeptide is provided. The recombinant polypeptide comprises: a polypeptide having substantial similarity to an extracellular domain

of a V-domain Ig Suppressor of T cell Activation (VISTA) (SEQ ID NO: 9 or 10) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11 or 12).

[0025] In an embodiment, the recombinant polypeptide comprises SEQ ID NO: 9 operably linked to SEQ ID NO: 11. In an embodiment, the recombinant polypeptide comprises SEQ ID NO: 10 operably linked to SEQ ID NO: 12.

[0026] In another aspect, a recombinant polypeptide is provided. The recombinant polypeptide comprises: a polypeptide having substantial similarity to an extracellular domain of B7-H4 (SEQ ID NO: 25) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).

[0027] In another aspect, a recombinant polypeptide is provided. The recombinant polypeptide comprises: a polypeptide having substantial similarity to an extracellular domain of PD-L1 (SEQ ID NO: 36) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).

[0028] In another aspect, a recombinant polypeptide is provided. The recombinant polypeptide comprises: a polypeptide having substantial similarity to an extracellular domain of ICOS-L (SEQ ID NO: 49) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).

[0029] In an embodiment, a recombinant polypeptide provided herein is in a soluble form.

[0030] In an aspect, a pentamerized polypeptide having T-cell inhibitory activity is provided. The pentamerized polypeptide having T-cell inhibitory activity comprises: five monomers, each of the monomers comprising: a polypeptide having substantial similarity to an extracellular domain of a V-domain Ig Suppressor of T cell Activation (VISTA) (SEQ ID NO: 9 or 10) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11 or 12).

[0031] In an embodiment, the recombinant polypeptide comprises SEQ ID NO: 9 operably linked to SEQ ID NO: 11. In an embodiment, the pentamerized polypeptide comprises SEQ ID NO: 10 operably linked to SEQ ID NO: 12. In an embodiment, the pentamerized polypeptide is in a soluble form. In an embodiment, the soluble form pentamerized polypeptide has increased T-cell inhibitory activity relative to a soluble dimerized polypeptide comprising an extracellular domain of VISTA (SEQ ID NO: 9 or 10). In an embodiment, the increased T-cell inhibitory activity comprises one or more of increased inhibition of T-cell activation and T-cell proliferation. In an embodiment, the soluble form

pentamerized polypeptide has increased immune inhibitory activity in vivo relative to a soluble dimerized polypeptide comprising an extracellular domain of VISTA (SEQ ID NO: 9 or 10). In an embodiment, the increased immune inhibitory activity comprises one or more of increased inhibition of cytokine secretion and cytotoxic lymphocyte (CTL) production.

[0032] In another aspect, a pentamerized polypeptide having T-cell inhibitory activity is provided. The pentamerized polypeptide having T-cell inhibitory activity comprises: five monomers, each of the monomers comprising: a polypeptide having substantial similarity to an extracellular domain of B7-H4 (SEQ ID NO: 25) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).

[0033] In an embodiment, the pentamerized polypeptide is in a soluble form. In an embodiment, the soluble form pentamerized polypeptide has increased T-cell inhibitory activity relative to a soluble dimerized polypeptide comprising an extracellular domain of B7-H4 (SEQ ID NO: 25). In an embodiment, the increased T-cell inhibitory activity comprises one or more of increased inhibition of T-cell activation and T-cell proliferation. In an embodiment, the soluble form pentamerized polypeptide has increased immune inhibitory activity in vivo relative to a soluble dimerized polypeptide comprising an extracellular domain of B7-H4 (SEQ ID NO: 25). In an embodiment, the increased immune inhibitory activity comprises one or more of increased inhibition of cytokine secretion and cytotoxic lymphocyte (CTL) production.

[0034] In another aspect, a pentamerized polypeptide having T-cell inhibitory activity is provided. The pentamerized polypeptide having T-cell inhibitory activity comprises: five monomers, each of the monomers comprising: a polypeptide having substantial similarity to an extracellular domain of PD-L1 (SEQ ID NO: 36) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).

[0035] In an embodiment, the pentamerized polypeptide is in a soluble form. In an embodiment, the soluble form pentamerized polypeptide has increased T-cell inhibitory activity relative to a soluble dimerized polypeptide comprising an extracellular domain of PD-L1 (SEQ ID NO: 36). In an embodiment, the increased T-cell inhibitory activity comprises one or more of increased inhibition of T-cell activation and T-cell proliferation. In an embodiment, the soluble form pentamerized polypeptide has increased immune inhibitory activity in vivo relative to a soluble dimerized polypeptide comprising an extracellular domain of PD-L1 (SEQ ID NO: 36). In an embodiment, the increased immune inhibitory activity

comprises one or more of increased inhibition of cytokine secretion and cytotoxic lymphocyte (CTL) production.

[0036] In another aspect, a pentamerized polypeptide having T-cell stimulatory activity is provided. The pentamerized polypeptide having T-cell stimulatory activity comprises: five monomers, each of the monomers comprising: a polypeptide having substantial similarity to an extracellular domain of ICOS-L (SEQ ID NO: 49) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).

[0037] In an embodiment, the pentamerized polypeptide is in a soluble form. In an embodiment, the soluble form pentamerized polypeptide has increased T-cell stimulatory activity relative to a soluble dimerized polypeptide comprising an extracellular domain of ICOS-L (SEQ ID NO: 49). In an embodiment, the increased T-cell stimulatory activity comprises one or more of increased stimulation of T-cell activation and T-cell proliferation. In an embodiment, the soluble form pentamerized polypeptide has increased immune stimulatory activity in vivo relative to a soluble dimerized polypeptide comprising an extracellular domain of ICOS-L (SEQ ID NO: 49). In an embodiment, the increased immune stimulatory activity comprises one or more of increased stimulation of cytokine secretion and cytotoxic lymphocyte (CTL) production. In an embodiment, the soluble form pentamerized polypeptide increases effector T-cells:regulatory T-cell ratios.

[0038] In an aspect, a pharmaceutical composition is provided. The pharmaceutical composition comprises: one or more of the polypeptides provided herein, the host cells provided herein, and the pentamerized polypeptides provided herein; and a pharmaceutically acceptable carrier, diluent, or excipient.

[0039] In an aspect, a method of eliciting a biological response in an individual in need thereof is provided. The method comprises: administering to the individual a therapeutically effective amount of a V-domain Ig Suppressor of T cell Activation (VISTA)-cartilage oligomeric matrix protein (COMP) fusion polypeptide (VISTA.COMP), the VISTA.COMP polypeptide having SEQ ID NO: 9 and being linked to SEQ ID NO: 11 or having SEQ ID NO: 10 and being linked to SEQ ID NO: 12, wherein the biological response is one or more of: suppression of T-cell activation; suppression of T-cell proliferation; decreased secretion by T-cells of one or more inflammatory cytokines; suppressed induction of cytotoxic T lymphocytes (CTLs); and an increase in T-cells with regulatory phenotypes. In an embodiment, the inflammatory cytokines comprise one or more of IL-2 and IFN γ .

[0040] In an aspect a method of eliciting a biological response in an individual in need thereof is provided. The method comprises administering to the individual a therapeutically

effective amount of a B7-H4-cartilage oligomeric matrix protein (COMP) fusion polypeptide (B7-H4.COMP), the B7-H4.COMP polypeptide having SEQ ID NO: 25 and being linked to SEQ ID NO: 11, wherein the biological response is one or more of: suppression of T-cell activation; suppression of T-cell proliferation; decreased secretion by T-cells of one or more inflammatory cytokines; suppressed induction of cytotoxic T lymphocytes (CTLs); and an increase in T-cells with regulatory phenotypes.

[0041] In an aspect, a method of eliciting a biological response in an individual in need thereof is provided. The method comprises administering to the individual a therapeutically effective amount of a PD-L1-cartilage oligomeric matrix protein (COMP) fusion polypeptide (PD-L1.COMP), the PD-L1.COMP polypeptide having SEQ ID NO: 36 and being linked to SEQ ID NO: 11, wherein the biological response is one or more of suppression of T-cell activation; suppression of T-cell proliferation; decreased secretion by T-cells of one or more inflammatory cytokines; suppressed induction of cytotoxic T lymphocytes (CTLs); and an increase in T-cells with regulatory phenotypes.

[0042] In an aspect, a method of eliciting a biological response in an individual in need thereof is provided. The method comprises administering to the individual a therapeutically effective amount of an ICOS-L-cartilage oligomeric matrix protein (COMP) fusion polypeptide (ICOS-L.COMP), the ICOS-L.COMP polypeptide having SEQ ID NO: 49 and being linked to SEQ ID NO: 11, wherein the biological response is one or more of stimulation of T-cell activation; stimulation of T-cell proliferation; increased secretion by T-cells of one or more inflammatory cytokines; increased induction of cytotoxic T lymphocytes (CTLs); and an increase in the effector T-cells:regulatory T-cell ratio within the tumor microenvironment. In an embodiment, the ICOS-L.COMP polypeptide is administered in combination with a checkpoint blocking molecule. In an embodiment, the ICOS-L.COMP polypeptide is administered simultaneously with or before or after the checkpoint blocking molecule. In an embodiment, the checkpoint blocking molecule is an anti-PD-1 antibody or an anti-CTLA-4 antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] The features of the invention will become more apparent in the following detailed description in which reference is made to the appended drawings wherein:

[0044] FIGs. 1A-G illustrate that pentameric VISTA.COMP, but not a dimeric VISTA-Fc, suppresses T-cell activation and proliferation as a soluble ligand in-vitro.

[0045] FIG. 1A depicts CFSE-labelled purified murine CD4⁺ T-cells that were activated by plate-bound anti-CD3 antibody (2.5 µg/mL) in the presence (dark grey) or absence (light

grey) of immobilized (left panel) or soluble (right panel) VISTA.Fc (10 µg/mL) for 48 hrs. VISTA.Fc suppressed the proliferation of CD4⁺ T-cells when immobilized, but not when added as a soluble ligand in the culture media.

[0046] FIG. 1B shows that recombinant VISTA.COMP was expressed as described in the Methods, and that the purity and pentameric status was confirmed by SDS-PAGE and Western blot in the presence or absence of a reducing agent (DTT). Reduced VISTA.COMP migrated as a single band ~50kDa and the disulphide-stabilized pentamer had an apparent mass of 250kDa.

[0047] FIG. 1C depicts results of a proliferation assay of CD4⁺ T-cells having undergone activation in the presence of coated (9 µg/mL, left panels) or soluble (12 µg/mL, right panels) VISTA.COMP (dark grey) or COMP (light grey). Soluble VISTA.COMP suppressed T-cell expansion (top, FSC & SSC scatter profiles) and proliferation (bottom, CFSE dilution).

[0048] FIGs. 1D and 1E depict analysis of culture medium harvested from CD4⁺ T-cells 48 and 72 hours post anti-CD3 activation in the presence of COMP or VISTA.COMP (10 µg/mL), in which IL-2 (FIG. 1D) and IFN γ (FIG. 1E) secretion were quantified by ELISA. In addition to proliferation, VISTA.COMP was found to significantly suppress IL-2 and IFN γ secretion from T-cells in-vitro (**p<0.005 relative to COMP control, n=3).

[0049] FIG. 1F shows that CFSE-labelled CD4⁺ T-cells were activated with immobilized anti-CD3 antibody at the indicated concentration in the presence of COMP (light grey) or VISTA.COMP (dark grey). VISTA.COMP suppression of T-cell proliferation was overcome by stronger levels of TCR stimulation.

[0050] FIG. 1G depicts results of allogenic MLC assays, in which addition of VISTA.COMP significantly suppressed CTL induction in responder cells from wild-type or CD200R1 ^{-/-} mice (n=3, *p<0.05 relative to CD200Fc).

[0051] FIG. 1H shows SDS-PAGE of the expressed pentameric form of human VISTA (hVISTA-COMP) and mVISTA-COMP.

[0052] FIG. 1I shows the expansion (top) and CFSE proliferation (bottom) of human T-cells stimulated in-vitro with Concanavalin A in the presence of soluble COMP or hVISTA.COMP. VISTA-COMP suppresses the proliferation induced in these T-cells.

[0053] FIG. 1J shows decreased upregulation of the T-cell activation marker CD25 in CD3⁺CD4⁺ and CD8⁺ T-cells when cultured in ConA in the presence of VISTA.COMP compared to COMP or VISTA-Fc.

[0054] FIGs. 2 A-H illustrate that VISTA.COMP (FIG. 2 C, dark grey) binds to a clonal T-cell line (unstained control in white). and suppresses its activation (FIGs. 2A-2B).

[0055] FIG. 2A depicts 2.10 clonal T-cells that were activated in culture with immobilized anti-CD3 antibody (3 µg/mL) in the presence of immobilized or soluble VISTA.Fc or VISTA.COMP (10 µg/mL) and proliferation was measured by pulsing cells with 3H-Thymidine in the last 6 hours of a 24hr culture. As observed with primary CD4⁺ T-cells, the pentameric VISTA.COMP suppressed proliferation both when immobilized or added soluble in culture media, whereas VISTA.Fc only exhibited suppressive activity when immobilized. (n=3, **p<0.01 relative to anti-CD3 stimulated control).

[0056] FIG. 2B depicts titration of soluble VISTA.COMP (dark grey) or VISTA.Fc (light grey) on 2.10 cells being activated as described in panel A. Data shows a lack of anti-proliferative function from cells treated with soluble VISTA.Fc at high concentrations (n=3, **p<0.01).

[0057] FIG. 2C depicts FACS analysis of biotinylated COMP, biotinylated VISTA.COMP, and VISTA.Fc (shaded histogram) binding to 2.10 clonal T-cells compared to unstained control (empty histograms).

[0058] FIG. 2D depicts anti-CD3 activated 2.10 T-cells that were treated with soluble VISTA.Fc or VISTA.COMP for 4 hours and the production of IL-2 measured by ICFC. Only VISTA.COMP significantly suppressed the number of IL-2 secreting cells (n=3, *p<0.05 relative to VISTA.Fc or non-treated).

[0059] FIG. 2E depicts 1x10⁶ 2.10 clonal T-cells that were cultured in a 6-well plate with immobilized anti-CD3 antibody and in the presence or absence of VISTA.COMP for 10 minutes. Proteins in complex with the T-cell receptor (TCR) were recovered by lysing the cells in each well and recovering the proteins adhered in each well (see solid phase immunoprecipitation (SPIP) in Methods). Recovered proteins were subsequently subject to immunoblot using anti-phosphotyrosine antibody (pY: clone 4G10). VISTA.COMP substantially diminished the phosphorylation of TCR complex proteins induced by TCR signalling.

[0060] FIGs. 2F-H depict stability of VISTA-Fc or VISTA.COMP binding to T-cell clones.

[0061] FIG. 2F depicts binding of VISTA-Fc or control-Fc (isotype control) to 2.10 T-cells, after one-step washing with FACS staining buffer.

[0062] FIG. 2G depicts binding of VISTA-Fc after two-step washing prior to FACS analysis. Loss of binding signal relative to A indicates a weak transient interaction of VISTA-Fc to the cell line.

[0063] FIG. 2H depicts binding of biotinylated VISTA.COMP to 2.10 cells and Jurkat cells is retained after two-step washing, indicating a more stable interaction of VISTA.COMP towards these cells.

[0064] FIGs. 3A-E illustrate that VISTA.COMP suppresses immune responses in-vivo.

[0065] FIG. 3A depicts a schematic representation of the skin allograft rejection model. On day 0, BALB/C animals were engrafted with skin from C57BL/6 mice and subsequently treated with VISTA.COMP (15 µg, I.V.) or PBS over the course of 15 days (arrows). Graft survival was monitored daily by a blinded investigator.

[0066] FIG. 3B depicts that treatment with VISTA.COMP significantly prolonged survival of skin allografts (n=6, *p<0.05 by Mann-Whitney U-Test).

[0067] FIG. 3C depicts treatment of VISTA.COMP 1hr prior to Con-A injection rescued C57BL/6 mice from lethal hepatic injury at 24h (n=4).

[0068] FIGs. 3D and E show that survival correlated with a significant decrease in serum TNFα (n=5, *p<0.05) (FIG. 3D) and IL6 (n=5, *p<0.05) (FIG. 3E) 3 hours post Con-A injection.

[0069] FIGs. 4A-B illustrate the influence of two different tags on VISTA.COMP activity.

[0070] FIG. 5A is a schematic of B7-H4.COMP and PD-L1.COMP pentamers.

[0071] FIG. 5B is a SDS-PAGE gel of Ni.NTA-purified reduced and oxidized B7-H4.COMP, PD-L1.COMP, VISTA.COMP (+control) and COMP constructs expressed in Expi293F cells.

[0072] FIG. 6A shows that CFSE-labelled purified murine CD4+ T-cells were activated by plate-bound anti-CD3 antibody (2C11) in the presence of the indicated soluble ligand for 72 hours. FACS analyses of CFSE dilution revealed that PD-L1.COMP, B7-H4.COMP and VISTA.COMP all suppressed the expansion (top) and proliferation (bottom) of T cells relative to COMP only or no ligand (-).

[0073] FIG. 6B shows FACS analyses of CFSE-labelled purified murine CD4+ T cells showing that unlike soluble dimeric VISTA.Fc, soluble VISTA.COMP can suppress T cell proliferation in response to CD3-TCR signaling.

[0074] FIG. 7A shows that IV injection of VISTA.COMP but not VISTA.Fc [every 3 days] blocks skin allograft rejection in mice until treatment was halted (asterisk).

[0075] FIG. 7B shows that treating C57BL/6 mice with VISTA.COMP by IP injection 1-hr prior to Con-A rescued 50% of them from lethal hepatic injury at 24h (n=13, p<0.05). VISTA.Fc was not effective. Moribund animals were classified as non-responders. Data was

pooled from three independent trials. VISTA.COMP treated mice exhibited a significant decrease in serum TNF α (n=5, *p<0.05) and IL6 (n=5, *p<0.05) 3 hours post Con-A injection.

[0076] FIG. 8A is a schematic diagram of human ICOS-L.COMP pentamers.

[0077] FIG. 8B is a western blot and SDS-PAGE showing purity and molecular weight of the ICOS-L.COMP pentamers.

[0078] FIG. 9A shows the binding of hICOS-L.COMP to both human and mouse ICOS, but not CD28 as measured by Biacore T200 surface plasmon resonance experiments.

[0079] FIG. 9B shows characterization of ICOS.Fc binding to human ICOS-L.COMP, hICOSL-Fc, and COMP by surface plasmon resonance. Strong avidity of human ICOS-L.COMP for ICOS is reflected in the slow off-rate observed (kd) and low dissociation constant (0.9nM), compared to 2.9nM for ICOSL-Fc.

[0080] FIG. 9C shows FACS analyses demonstrating the binding of FITC-labelled ICOS-L.COMP binding to human CD3+CD4+ and CD3+CD4- T-cells.

[0081] FIG. 9D shows FACS analyses demonstrating the competitive displacement of hICOS-L.Fc binding to activated human CD3+ T-cells by human ICOS-L.COMP.

[0082] FIG. 9E shows FACS analyses demonstrating the competitive displacement of mICOSL-Fc binding to activated mouse CD4+ T-cells by hICOS-L.COMP.

[0083] FIG. 10A shows CFSE-based T cell proliferation assays that demonstrate the robust co-stimulation of both human CD4+ and CD8+ T-cells with soluble ICOS-L.COMP.

[0084] FIG. 10B shows a FACS analysis demonstrating that the activation of T cells with human ICOS-L.COMP results in the increased expression of CD25 on T cells (grey profile).

[0085] FIG. 10C shows CFSE-based T-cell proliferation demonstrating that soluble hICOS-L.COMP, but not ICOSL-Fc co-stimulates CD3+CD4+ human T-cells isolated from umbilical cord blood.

[0086] FIG. 10D shows a FACS analysis demonstrating that in the absence of anti-CD3 induced T-cell receptor signalling, ICOSL-COMP does not induce proliferation of human T-cells isolated from umbilical cord blood.

[0087] FIG. 10E shows CFSE T-cell proliferation assays demonstrating that soluble hICOSL-COMP, but not ICOSL-Fc co-stimulates CD3+CD4+ and CD3+CD4- T-cell proliferation in combination with anti-CD3 or anti-CD3/anti-CD28 induced signalling. T-cells were isolated from adult donor PBMCs.

[0088] FIG. 10F shows the cytokine secretion (IL2, IL6, IFN γ , TNF α , IL10) after 72 hours of stimulation of CD3+ T-cells (from adult PBMCs) with anti-CD3 in the presence of soluble COMP, ICOSL-COMP or ICOSL-Fc.

[0089] FIG. 11A is a schematic diagram detailing administration of compounds in in-vivo MC38 tumor model experiments.

[0090] FIG. 11B shows that ICOS-L.COMP acts in synergy with anti-PD-1 mAb to induce protective anti-tumor immunity in C57Bl/6 mice bearing established, subcutaneous murine colorectal MC38 tumor (therapeutic model).

[0091] FIG. 11C shows individual mice tumor volume profiles as a function of time.

[0092] FIG. 12A shows that ICOS-L.COMP monotherapy did not reduce the tumor growth in the MC38 tumor model.

[0093] FIG. 12B shows that anti-PD-1 treatment of MC38 tumor bearing animals leads to an increase in the expression of ICOS in intratumoral CD45+CD4+ and CD45+CD8+ T-cells.

[0094] FIG. 12C shows the TIL profiles in treated MC38 tumor bearing animals. The combination of anti-PD-1 and ICOS-L.COMP led to a significant increase in the abundance of CD45+CD4+FoxP3- cells among the TIL population.

[0095] FIG. 12D shows the TIL profiles in treated MC38 tumor bearing animals. The combination of anti-PD-1 and ICOS-L.COMP had no change in the abundance of CD45+CD4+FoxP3+ T-regulator cells among the TIL population.

[0096] FIG. 13 shows staining of 24h anti-CD3 activated or naive 2.10 T-cells with biotinylated PD-L1.COMP, B7-H4.COMP, or COMP. COMP shows negligible non-specific binding to this T-cell line compared to PD-L1 or B7-H4.COMP.

[0097] FIG. 14 shows that immobilized and soluble B7-H4.COMP suppresses the proliferation 2.10 T-cells undergoing activation with immobilized anti-CD3 antibody.

[0098] FIG. 15 shows that soluble B7-H4.COMP (10ug/mL) substantially suppresses the expansion (top) and division (bottom) of primary murine CD4+ T-cells undergoing 72h of anti-CD3 induced activation in-vitro.

[0099] FIG. 16 shows that soluble B7-H4.COMP (10ug/mL) inhibits IL-2 cytokine secretion from primary murine CD4+ T-cells undergoing 48h of anti-CD3 induced activation in-vitro.

DETAILED DESCRIPTION OF THE NON-LIMITING EXEMPLARY EMBODIMENTS

[00100] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[00101] V-domain Immunoglobulin suppressor of T-cell activation (VISTA) is a recently-discovered immune checkpoint ligand that functions to suppress T-cell activity. Other immune checkpoint ligands include B7-H4 and PD-L1. Activation of this immune checkpoint pathway in a subject has therapeutic potential, at least because it may reduce inflammatory responses in the subject by inhibiting T-cell activity. Conversely, ligands that stimulate T-cell activity, such as ICOS-L, have immune-boosting therapeutic potential, such as in cancer immunotherapy.

[00102] A dimeric construct of the IgV domain of VISTA (VISTA-Fc) was shown to suppress T-cell activation in-vitro. However, this effect required immobilization of VISTA-Fc to a solid substrate. Immobilization-dependent activity suggests that the efficacy of VISTA-Fc as a VISTA-receptor agonist in-vivo may be limited.

[00103] Provided herein is a pentameric polypeptide and monomers that make up same, each of the monomers containing an extracellular domain of VISTA, B7-H4, PD-L1 or ICOS-L genetically fused or linked to the pentamerization domain of cartilage oligomeric matrix protein (COMP).

[00104] COMP is a 524 kDa homopentamer of five subunits which consists of an N-terminal heptad repeat region (cc) followed by four epidermal growth factor (EGF)-like domains (EF), seven calcium-binding domains (T3) and a C-terminal globular domain (TC). The COMP pentamerization domain used herein is a 45-amino acid long sequence that spontaneously assembles into a bundle of 5 alpha-helices arranged in a parallel orientation and stabilized by disulphide bridges. Previously, a pro-angiogenic factor angiopoietin 1 that was fused to the COMP pentamerization domain (COMP-Ang1) showed increased stability relative to native Ang1, which lead to an increased induction of angiogenesis in-vivo²¹.

[00105] The inventors have generated engineered nucleic acids, engineered polypeptides, and engineered pentamerized polypeptides, human and murine, each of which include sequences of a VISTA, B7-H4, PD-L1 or ICOS-L extracellular domain operably linked to the pentamerization domain of COMP. By "extracellular domain" (or "ECD"), we mean the extracellular region of the polypeptide, or the nucleic acid that codes for it, that contains one or more Ig-type domains, which play a role in efficient binding between ligand and receptor. The ECD of VISTA, B7-H4, PD-L1 and ICOS-L comprises an IgV domain. The

ECD of B7-H4, PD-L1 and ICOS-L also comprises an IgC domain. Engineered mRNAs corresponding to the engineered nucleic acids and/or polypeptides provided herein are also contemplated herein.

[00106] Appendix 1 provides nucleic acid and polypeptide sequences for use in preparing a VISTA, B7-H4, PD-L1 or ICOS-L extracellular domain operably linked to the pentamerization domain of COMP.

[00107] In an embodiment, a recombinant nucleic acid having a nucleic acid sequence encoding an extracellular domain of VISTA, B7-H4, PD-L1 or ICOS-L linked to a pentamerization domain of COMP is provided. In some embodiments, the nucleic acid encoding an extracellular IgV-containing domain VISTA has substantial similarity to SEQ ID NO: 1 (the human IgV-containing domain of VISTA) or SEQ ID NO: 2 (the mouse IgV-containing domain of VISTA). In some embodiments, the nucleic acid encoding the extracellular domain of B7-H4 has substantial similarity to SEQ ID NO: 26. In some embodiments, the nucleic acid encoding the extracellular domain of PD-L1 has substantial similarity to SEQ ID NO: 37. In some embodiments, the nucleic acid encoding the extracellular domain of ICOS-L has substantial similarity to SEQ ID NO: 48. In some embodiments, the nucleic acid encoding the pentamerization domain of COMP has substantial similarity to SEQ ID NO: 3 (the human pentamerization domain of COMP) or SEQ ID NO: 4 (the mouse pentamerization domain of COMP).

[00108] By “substantial similarity” in sequence, we mean sequences that are identical to or variants of the sequences provided herein, and encompass, or encode for a polypeptide that encompasses, the biological activity described herein.

[00109] For example, for nucleic acid sequences, substantially similar sequences include conservative variants that, because of the degeneracy of the genetic code, encode the amino acid sequence of one of the polypeptides provided herein. Variant nucleotide sequences include synthetically derived nucleotide sequences. Generally, variants of a particular nucleotide sequence of the invention will have at least at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to one of the nucleotide sequences provided herein, as determined by sequence alignment programs known in the art, and using default parameters. In some preferred embodiments, substantially similar sequences are identical to the sequence referred to. In some preferred embodiments, the nucleic acid sequence is codon optimized for use in a genetic construct (e.g., for use in a plasmid).

[00110] Variant polypeptides encompassed by the present invention are biologically active, that is they continue to possess the biological activity of the pentamerized

polypeptide described herein. Such variants may result from, for example, genetic polymorphism or from human manipulation. Biologically active variants of a polypeptide of the invention will have at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to one of the amino acid sequences provided herein as determined by sequence alignment programs known in the art using default parameters.

[00111] In an embodiment, the recombinant nucleic acid comprises SEQ ID NO: 1 operably linked to SEQ ID NO: 3 (i.e., the human IgV-containing domain of VISTA linked to the human pentamerization domain of COMP). In an embodiment, the recombinant nucleic acid comprises SEQ ID NO: 2 operably linked to SEQ ID NO: 4 (i.e., the mouse IgV-containing domain of VISTA linked to the mouse pentamerization domain of COMP).

[00112] A nucleic acid molecule is operably linked to another nucleic acid molecule when it is placed into a functional relationship with another nucleic acid molecule. For example, two nucleic acid molecules are operably linked when they are joined such that the amino acid sequences encoded by the two nucleic acid molecules provide for proper translation. Such nucleic acids may be operably linked through a linker sequence. Linker sequences suitable for use with the recombinant nucleic acids disclosed herein may be determined by a person of skill in the art. In some preferred embodiments, the linker sequence will be engineered to encode a somewhat flexible peptide or polypeptide (for example, it may be glycine rich).

[00113] In an aspect, a recombinant messenger ribonucleic acid (mRNA) having an mRNA sequence encoding an extracellular domain of VISTA linked to a pentamerization domain of COMP is provided. In some embodiments, the mRNA encoding the extracellular domain of VISTA has substantial similarity to SEQ ID NO: 5 (the human IgV-containing domain of VISTA) or SEQ ID NO: 6 (the mouse IgV-containing domain of VISTA). In some embodiments, the mRNA encoding the pentamerization domain of COMP has substantial similarity to SEQ ID NO: 7 (the human pentamerization domain of COMP) or SEQ ID NO: 8 (the mouse pentamerization domain of COMP).

[00114] In an embodiment, the recombinant mRNA comprises SEQ ID NO: 5 operably linked to SEQ ID NO: 7 (i.e., the human IgV-containing domain of VISTA linked to the human pentamerization domain of COMP). In an embodiment, the recombinant nucleic acid comprises SEQ ID NO: 6 operably linked to SEQ ID NO: 8 (i.e., the mouse IgV-containing domain of VISTA linked to the mouse pentamerization domain of COMP). Linker sequences suitable for use with the recombinant nucleic acids disclosed herein may be determined by a person of skill in the art. In some preferred embodiments, the linker sequence will be

engineered to encode a somewhat flexible peptide or polypeptide (for example, it may be glycine rich).

[00115] In an aspect, a recombinant messenger ribonucleic acid (mRNA) having an mRNA sequence encoding an extracellular domain of B7-H4 linked to a pentamerization domain of COMP is provided. In some embodiments, the mRNA encoding the extracellular domain of B7-H4 has substantial similarity to SEQ ID NO: 27 (the human ECD of B7-H4). In some embodiments, the mRNA encoding the pentamerization domain of COMP has substantial similarity to SEQ ID NO: 7 (the human pentamerization domain of COMP).

[00116] In an aspect, a recombinant messenger ribonucleic acid (mRNA) having an mRNA sequence encoding an extracellular domain of PD-L1 linked to a pentamerization domain of COMP is provided. In some embodiments, the mRNA encoding the extracellular domain of VISTA has substantial similarity to SEQ ID NO: 62 (the human ECD of PD-L1). In some embodiments, the mRNA encoding the pentamerization domain of COMP has substantial similarity to SEQ ID NO: 7 (the human pentamerization domain of COMP).

[00117] In an aspect, a recombinant messenger ribonucleic acid (mRNA) having an mRNA sequence encoding an extracellular domain of ICOS-L linked to a pentamerization domain of COMP is provided. In some embodiments, the mRNA encoding the extracellular domain of VISTA has substantial similarity to SEQ ID NO: 61 (the human ECD of ICOS-L). In some embodiments, the mRNA encoding the pentamerization domain of COMP has substantial similarity to SEQ ID NO: 7 (the human pentamerization domain of COMP).

[00118] In an aspect, a recombinant polypeptide having an amino acid sequence encoding an extracellular domain of VISTA, B7-H4, PD-L1 or ICOS-L linked to a pentamerization domain of COMP is provided. In some embodiments, the amino acid sequence encoding the extracellular IgV-containing domain of VISTA has substantial similarity to SEQ ID NO: 9 (the human IgV-containing domain of VISTA) or SEQ ID NO: 10 (the mouse IgV-containing domain of VISTA). In some embodiments, the amino acid sequence encoding the extracellular domain of B7-H4 has substantial similarity to SEQ ID NO: 25. In some embodiments, the amino acid sequence encoding the extracellular domain PD-L1 has substantial similarity to SEQ ID NO: 36. In some embodiments, the amino acid sequence encoding the extracellular domain ICOS-L has substantial similarity to SEQ ID NO: 49. In some embodiments, the amino acid encoding the pentamerization domain of COMP has substantial similarity to SEQ ID NO: 11 (the human pentamerization domain of COMP) or SEQ ID NO: 12 (the mouse pentamerization domain of COMP).

[00119] As used herein, "link" means covalently or non-covalently associating one polypeptide to another polypeptide, regardless of the method of association. In one

embodiment the association is a covalent association, such as a peptide bond. For example, a peptide having an amino acid sequence encoding an extracellular domain of VISTA, B7-H4, PD-L1 or ICOS-L of the invention can be linked to a pentamerization domain of COMP of the invention. Examples of such linkers are known in the art and are described for example, in Chen et. al (Reference 38). In an embodiment, an extracellular domain of VISTA, B7-H4, PD-L1 or ICOS-L of the invention can be linked to a pentamerization domain of COMP by forming a fusion protein between the extracellular domain of VISTA, B7-H4, PD-L1 or ICOS-L and the pentamerization domain of COMP. Such fusion proteins can be produced in host cells using expression vectors encoding VISTA, B7-H4, PD-L1 or ICOS-L ECDs and the pentamerization domain of COMP according to standard methods known in the art and as described herein.

[00120] In an embodiment, the recombinant polypeptide comprises SEQ ID NO: 9 operably linked to SEQ ID NO: 11 (i.e., the human extracellular IgV-containing domain of VISTA linked to the human pentamerization domain of COMP). In an embodiment, the recombinant nucleic acid comprises SEQ ID NO: 10 operably linked to SEQ ID NO: 12 (i.e., the mouse extracellular IgV-containing domain of VISTA linked to the mouse pentamerization domain of COMP). Linker sequences suitable for use with the recombinant nucleic acids disclosed herein may be determined by a person of skill in the art. In some preferred embodiments, the linker sequence will be engineered to encode a somewhat flexible peptide or polypeptide (for example, it may be glycine rich).

[00121] Herein, in some embodiments, the recombinant polypeptide may also be referred to as a recombinant protein, an engineered protein, or a fusion protein. By "fusion protein", we mean a protein generated by joining two or more genes which originally coded for separate polypeptides. Translation of this fusion gene results in a single polypeptide with functional properties derived from each of the original polypeptides.

[00122] In some embodiments the nucleic acid or polypeptide of the invention may include an N-terminal leader sequence to enable secretion of the recombinant protein and/or a Histidine or other affinity tag for purification purposes. Methods for introducing an N-terminal leader sequence and/or a Histidine or other affinity tag are known in the art.

[00123] In an embodiment, the recombinant polypeptide is provided in a soluble form. As used herein, "soluble" means without immobilization on a solid substrate or a solid surface. In an embodiment, the activity of the recombinant polypeptide is substrate immobilization-independent (i.e., activity does not depend on the recombinant polypeptide being immobilized on a solid substrate or solid surface).

[00124] In an embodiment, an expression vector comprising a recombinant polypeptide disclosed herein is provided. In some embodiments, the expression vector further comprises at least one control sequence. By "control sequences", we mean one or more sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable include, for example, promoters, polyadenylation signals, and/or enhancers. Methods and tools for generating an expression vector housing a recombinant polypeptide are known in the art and may be suitable for generating the expression vectors provided herein.

[00125] In an aspect, a host cell comprising an expression vector disclosed herein is provided. For example, the host cell may be HEK-293 or a HEK-293 derivative, CHO or a CHO derivative, or NS01 or an NS01 derivative. Methods and tools for generating a host cell housing an expression vector are known in the art and may be suitable for generating the host cells provided herein. In an embodiment, an engineered cell line comprising VISTA and COMP, B7-H4 and COMP, PD-L1 and COMP, or ICOS-L and COMP genetic material (as provided herein) integrated into the genome thereof is provided.

[00126] In an aspect, a pentamerized polypeptide having T-cell modulating activity is provided. The pentamerized polypeptide includes five monomers, each of the monomers comprising: a polypeptide having substantial similarity to an extracellular domain of a V-domain Ig Suppressor of T cell Activation (VISTA) (SEQ ID NO: 9 or 10); a polypeptide having substantial similarity to an extracellular domain of B7-H4 (SEQ ID NO: 25); a polypeptide having substantial similarity to an extracellular domain of PD-L1 (SEQ ID NO: 36); or a polypeptide having substantial similarity to an extracellular domain of ICOS-L (SEQ ID NO: 49); linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11 or 12).

[00127] In an embodiment, the pentamerized polypeptide comprises SEQ ID NO: 9 operably linked to SEQ ID NO: 11.

[00128] In an embodiment, the pentamerized polypeptide comprises SEQ ID NO: 10 operably linked to SEQ ID NO: 12.

[00129] In some embodiments, the pentamerized polypeptide is in a soluble form. In contrast to the dimerized VISTA-Fc, the pentamerized VISTA.COMP, B7-H4.COMP and PD-L1.COMP provided herein is biologically active in its soluble form. A skilled person will appreciate the possible advantages of an agent that is biologically active in its soluble relative to one that is active only in its immobilized form. For example, a soluble VISTA-receptor agonist, B7-H4-receptor agonist or PD-1 receptor agonist may exhibit increased

activity in vivo, relative to a dimeric version (VISTA-Fc, B7-H4-Fc or PD-L1-Fc) which may require binding and clustering on accessory cells to induce immunosuppression.

[00130] In some embodiments, the soluble form pentamerized polypeptide has increased T-cell inhibitory activity relative to a soluble dimerized polypeptide comprising the IgV-containing domain of VISTA (SEQ ID NO: 9 or 10) (e.g., relative to VISTA-Fc) or relative to a soluble dimerized polypeptide comprising the ECD of B7-H4 (e.g., relative to B7-H4-Fc), or relative to a soluble dimerized polypeptide comprising the ECD of PD-L1 (e.g., relative to PD-L1-Fc). The T-cell inhibitory activity of the soluble form pentamerized polypeptide may be increased by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% relative to the T-cell inhibitory activity of a soluble dimerized polypeptide comprising the IgV-containing domain of VISTA (SEQ ID NO: 9 or 10) (e.g., relative to VISTA-Fc) or relative to a soluble dimerized polypeptide comprising the ECD of B7-H4 (e.g., relative to B7-H4-Fc), or relative to a soluble dimerized polypeptide comprising the ECD of PD-L1 (e.g., relative to PD-L1-Fc). In some embodiments, the increased T-cell inhibitory activity comprises one or more of increased inhibition of T-cell activation and T-cell proliferation. Methods for determining T-cell inhibitory activity, T-cell activation and T-cell proliferation are known in the art and are described, for example, herein.

[00131] In some embodiments, the soluble form VISTA.COMP, B7-H4.COMP or PD-L1.COMP pentamerized polypeptide has increased immune inhibitory activity in vivo relative to a soluble dimerized polypeptide comprising an extracellular domain of VISTA (SEQ ID NO: 9 or 10) (e.g., relative to VISTA-Fc) or relative to a soluble dimerized polypeptide comprising the ECD of B7-H4 (e.g., relative to B7-H4-Fc), or relative to a soluble dimerized polypeptide comprising the ECD of PD-L1 (e.g., relative to PD-L1-Fc). The immune inhibitory activity of the soluble form pentamerized polypeptide may be increased by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% relative to the immune inhibitory activity of a soluble dimerized polypeptide comprising the IgV-containing domain of VISTA (SEQ ID NO: 9 or 10) (e.g., relative to VISTA-Fc) or relative to a soluble dimerized polypeptide comprising the ECD of B7-H4 (e.g., relative to B7-H4-Fc), or relative to a soluble dimerized polypeptide comprising the ECD of PD-L1 (e.g., relative to PD-L1-Fc). For example, the increased immune inhibitory activity may include one or more of increased inhibition of cytokine secretion (e.g., IL-2 and/or IFN γ) and increased inhibition of cytotoxic lymphocyte (CTL) production. Methods for determining immune inhibitory activity, cytokine secretion and inhibition of cytotoxic lymphocyte (CTL) production are known in the art and are described, for example, herein. For example, the increased immune inhibitory activity may include suppression of inflammatory responses in-vivo, as

demonstrated in the Examples section by data indicating prolongation of murine skin allograft survival, and protection of mice from lethal acute hepatitis.

[00132] In some embodiments, the soluble form ICOS-L.COMP pentamerized polypeptide has increased T-cell stimulatory activity relative to a soluble dimerized polypeptide comprising the ECD of ICOS-L (SEQ ID NO: 49) (e.g., relative to ICOS-L-Fc). The T-cell stimulatory activity of the soluble form pentamerized polypeptide may be increased by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 or more fold relative to the T-cell stimulatory activity of a soluble dimerized polypeptide comprising the ECD of ICOS-L (SEQ ID NO: 49) (e.g., relative to ICOS-L-Fc). In some embodiments, the increased T-cell stimulatory activity comprises one or more of increased stimulation of T-cell activation and T-cell proliferation. Methods for determining T-cell stimulatory activity, T-cell activation and T-cell proliferation are known in the art and are described, for example, herein.

[00133] In some embodiments, the soluble form ICOS-L.COMP pentamerized polypeptide has increased immune stimulatory activity in vivo relative to a soluble dimerized polypeptide comprising an extracellular domain of ICOS-L (SEQ ID NO: 49) (e.g., relative to ICOS-L-Fc). The immune stimulatory activity of the soluble form pentamerized polypeptide may be increased by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 or more fold relative to the immune stimulatory activity of a soluble dimerized polypeptide comprising the ECD of ICOS-L (SEQ ID NO: 49) (e.g., relative to ICOS-L-Fc). For example, the increased immune stimulatory activity may include one or more of increased cytokine secretion and increased cytotoxic lymphocyte (CTL) production. Methods for determining immune stimulatory activity, cytokine secretion and cytotoxic lymphocyte (CTL) production are known in the art and are described, for example, herein.

[00134] In some embodiments, the soluble form ICOS-L.COMP pentamerized polypeptide has an increase in the effector T-cells:regulatory T-cell ratios relative to a soluble dimerized polypeptide comprising an extracellular domain of ICOS-L (SEQ ID NO: 49) (e.g., relative to ICOS-L-Fc). The effector T-cells:regulatory T-cell ratios of the soluble form pentamerized polypeptide may be increased by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold relative to an appropriate control. Methods for determining effector T-cells:regulatory T-cell ratios are known in the art and are described, for example, herein.

[00135] In some embodiments, the soluble form of the VISTA.COMP, B7-H4.COMP or PD-L1.COMP pentamerized polypeptide provided herein exhibits activity as an agonist of the putative VISTA receptor, the putative B7-H4 receptor or the PD-1 receptor, respectively, in vitro and/or in vivo. By “agonist”, we mean an agent that binds to a receptor and activates the receptor thereby effecting a biological response.

[00136] In an embodiment, a pharmaceutical composition comprising one or more of the polypeptides, host cells, or pentamerized polypeptides disclosed herein and a pharmaceutically acceptable carrier, diluent, or excipient is provided herein.

[00137] The polypeptides or VISTA.COMP, B7-H4.COMP or PD-L1.COMP pentamerized polypeptides of the invention can be formulated in various ways using art recognized techniques. In some embodiments, the therapeutic compositions of the invention can be administered neat or with a minimum of additional components while others may optionally be formulated to contain suitable pharmaceutically acceptable carriers. As used herein, “pharmaceutically acceptable carriers” comprise excipients, vehicles, adjuvants and diluents that are well known in the art and can be available from commercial sources for use in pharmaceutical preparation (see, e.g., Gennaro (2003) Remington: The Science and Practice of Pharmacy with Facts and Comparisons: Drugfacts Plus, 20th ed., Mack Publishing; Ansel et al. (2004) Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th ed., Lippencott Williams and Wilkins; Kibbe et al. (2000) Handbook of Pharmaceutical Excipients, 3rd ed., Pharmaceutical Press.)

[00138] Suitable pharmaceutically acceptable carriers comprise substances that are relatively inert and can facilitate administration of polypeptides, host cells or pentamerized polypeptides or can aid processing of the polypeptides, host cells or pentamerized polypeptides into preparations that are pharmaceutically optimized for delivery to the site of action.

[00139] Such pharmaceutically acceptable carriers include agents that can alter the form, consistency, viscosity, pH, tonicity, stability, osmolarity, pharmacokinetics, protein aggregation or solubility of the formulation and include buffering agents, wetting agents, emulsifying agents, diluents, encapsulating agents and skin penetration enhancers. Certain non-limiting examples of carriers include saline, buffered saline, dextrose, arginine, sucrose, water, glycerol, ethanol, sorbitol, dextran, sodium carboxymethyl cellulose and combinations thereof. Polypeptides, host cells or pentamerized polypeptides for systemic administration may be formulated for enteral, parenteral or topical administration. In certain embodiments the disclosed compositions will be formulated for intravenous administration and will preferably be infused using an IV container (e.g. an IV drip bag). Indeed, all three types of

formulation may be used simultaneously to achieve systemic administration of the active ingredient. Excipients as well as formulations for parenteral and nonparenteral drug delivery are set forth in Remington: The Science and Practice of Pharmacy (2000) 20th Ed. Mack Publishing.

[00140] In an aspect, a method of eliciting a biological response in an individual in need thereof is provided. The method involves administering to the individual a therapeutically effective amount of: a VISTA-COMP fusion polypeptide (VISTA.COMP) comprising a) SEQ ID NO: 9 operably linked to SEQ ID NO: 11, or b) SEQ ID NO: 10 operably linked to SEQ ID NO: 12; a B7-H4-COMP fusion polypeptide (B7-H4.COMP) comprising SEQ ID NO: 25 operably linked to SEQ ID NO: 11; or a PD-L1-COMP fusion polypeptide (PD-L1.COMP) comprising SEQ ID NO: 36 operably linked to SEQ ID NO: 11. In this method, the biological response is one or more of: suppression of T-cell activation; suppression of T-cell proliferation; decreased secretion by T-cells of one or more inflammatory cytokines; suppressed induction of cytotoxic T lymphocytes (CTLs); and an increase in T-cells with regulatory phenotypes.

[00141] The suppression of T-cell activation in the individual administered the soluble form VISTA.COMP, B7-H4.COMP or PD-L1.COMP pentamerized polypeptide may be 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% relative to an appropriate control (for example, an individual receiving no polypeptide or a subject receiving soluble dimerized polypeptide). The suppression of T-cell proliferation in the individual administered the soluble form pentamerized polypeptide may be 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% relative to an appropriate control (for example, an individual receiving no polypeptide or a subject receiving soluble dimerized polypeptide). The secretion by T-cells of one or more inflammatory cytokines in the individual administered the soluble form pentamerized polypeptide may be decreased by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% relative to an appropriate control (for example, an individual receiving no polypeptide or a subject receiving soluble dimerized polypeptide). The induction of cytotoxic T lymphocytes (CTLs) in the individual administered the soluble form pentamerized polypeptide may be suppressed by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% relative to an appropriate control (for example, an individual receiving no polypeptide or a subject receiving soluble dimerized polypeptide). The increase in T-cells with regulatory phenotypes in the individual administered the soluble form pentamerized polypeptide may be increased by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold relative to an appropriate control

(for example, an individual receiving no polypeptide or a subject receiving soluble dimerized polypeptide).

[00142] Methods for determining suppression of T-cell activation; suppression of T-cell proliferation; decreased secretion by T-cells of one or more inflammatory cytokines; suppressed induction of cytotoxic T lymphocytes (CTLs); and an increase in T-cells with regulatory phenotypes are known in the art and are described, for example, herein.

[00143] In an embodiment, a method of eliciting a biological response in an individual in need thereof is provided. The method involves administering to the individual a therapeutically effective amount of: an ICOS-L-COMP fusion polypeptide (ICOS-L.COMP) comprising SEQ ID NO: 49 operably linked to SEQ ID NO: 11. In this method, the biological response is one or more of: enhancement of T-cell activation; enhancement of T-cell proliferation; increased secretion by T-cells of one or more inflammatory cytokines; and enhanced induction of cytotoxic T lymphocytes (CTLs), and increases in the effector T-cells:regulatory T-cell ratios.

[00144] T-cell activation in the individual administered the soluble form ICOS-L.COMP pentamerized polypeptide may be increased or enhanced by 5%, 10%, 15%, 20%, 25%, 30% , 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 or more fold relative to an appropriate control (for example, an individual receiving no polypeptide or a subject receiving soluble dimerized polypeptide (e.g., ICOS-L-Fc). T-cell proliferation in the individual administered the soluble form pentamerized polypeptide may be increased or enhanced by 5%, 10%, 15%, 20%, 25%, 30% , 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 or more fold relative to an appropriate control (for example, an individual receiving no polypeptide or a subject receiving soluble dimerized polypeptide). The secretion by T-cells of one or more inflammatory cytokines in the individual administered the soluble form pentamerized polypeptide may be increased by 5%, 10%, 15%, 20%, 25%, 30% , 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 or more fold relative to an appropriate control (for example, an individual receiving no polypeptide or a subject receiving soluble dimerized polypeptide). The induction of cytotoxic T lymphocytes (CTLs) in the individual administered the soluble form pentamerized polypeptide may be increased or enhanced by 5%, 10%, 15%, 20%, 25%, 30% , 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold relative to an appropriate control (for example, an individual receiving no polypeptide or a subject receiving soluble dimerized polypeptide). The increase in the

effector T-cells:regulatory T-cell ratios in the individual administered the soluble form pentamerized polypeptide may be increased by 5%, 10%, 15%, 20%, 25%, 30% , 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold relative to an appropriate control (for example, an individual receiving no polypeptide or a subject receiving soluble dimerized polypeptide).

[00145] Methods for determining enhancement of T-cell activation; enhancement of T-cell proliferation; increased secretion by T-cells of one or more inflammatory cytokines; and enhanced induction of cytotoxic T lymphocytes (CTLs), and increases in the effector T-cells:regulatory T-cell ratios are known in the art and are described, for example, herein.

[00146] By "therapeutically effective amount", we mean an amount effective to achieve the intended purpose (i.e., an amount sufficient to elicit a biological response in an individual in need thereof). Determination of a therapeutically effective amount is well within the capability of those skilled in the art.

[00147] The ICOS-L.COMP polypeptide may administered in combination with a checkpoint blocking molecule. As used herein a checkpoint blocking molecule is an agent that is capable of blocking immunoinhibitory signals to improve anti-tumor immune responses. The ICOS-L.COMP polypeptide can be administered simultaneously with, or before, or after the checkpoint blocking molecule. In an embodiment, the checkpoint blocking molecule is an inhibitor - for example an antagonistic antibody against PD-1, PD-L1, CTLA-4, LAG3, VISTA or TIM3.

[00148] Desired outcomes of the disclosed combinations are quantified by comparison to a control or baseline measurement. As used herein, relative terms such as "improve," "increase," or "reduce" indicate values relative to a control, such as a measurement in the same individual prior to initiation of treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the soluble form pentamerized polypeptides described herein but in the presence of other therapeutic moiety(ies) such as standard of care treatment. A representative control individual is an individual afflicted with the same condition as the individual being treated.

[00149] Changes or improvements in response to therapy (whether additive or synergistic) may prove to be statistically significant. As used herein, the term "significance" or "significant" relates to a statistical analysis of the probability that there is a non-random association between two or more measured responses. To determine whether or not a relationship is "significant" or has "significance," a "p-value" can be calculated. P-values that fall below a user-defined cut-off point are regarded as significant. For the purposes of the

instant invention a p-value less than or equal to 0.1, less than 0.05, less than 0.01, less than 0.005, or less than 0.001 may be regarded as significant.

[00150] A synergistic therapeutic effect may be an effect of at least about two-fold greater than the therapeutic effect elicited by a single therapeutic moiety, or the sum of the therapeutic effects elicited by the single therapeutic moieties) of a given combination, or at least about five-fold greater, or at least about ten-fold greater, or at least about twenty-fold greater, or at least about fifty-fold greater, or at least about one hundred-fold greater. A synergistic therapeutic effect may also be observed as an increase in therapeutic effect of at least 10% compared to the therapeutic effect elicited by a single therapeutic, or the sum of the therapeutic effects elicited by the single therapeutic moieties of a given combination, or at least 20%, or at least 30%, or at least 40%, or at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 100%, or more. A synergistic effect is also an effect that permits reduced dosing of therapeutic agents when they are used in combination.

[00151] The particular dosage regimen, i.e., dose, timing and repetition, will depend on the individual subject, as well as empirical considerations such as pharmacokinetics (e.g., half-life, clearance rate, etc.). Determination of the frequency of administration may be made by persons skilled in the art, such as an attending physician based on considerations of the condition and severity of the condition being treated, age and general state of health of the subject being treated and the like. Frequency of administration may be adjusted over the course of therapy based on assessment of the efficacy of the selected composition and the dosing regimen. Such assessment can be made on the basis of markers of the specific disease, disorder or condition or assessments of the individuals wellbeing (as measured using quality of life assessments, activities of daily living, etc.).

EXAMPLES

Example 1: VISTA.COMP Methods

Recombinant Protein Expression and Purification

[00152] VISTA.Fc was produced by cloning a synthetic dsDNA coding for the extracellular domain (ECD; residues 16-194 of SEQ ID NO: 10) of murine VISTA upstream of the human IgG-1 Fc region (GeneArt; Thermo Fisher Scientific) into the pcDNA-3.4 expression plasmid (Thermo Fisher Scientific). The plasmid encoding the murine VISTA.COMP (SEQ ID NO: 14) gene was similarly generated by inserting a synthetic dsDNA coding for the ECD of VISTA, flanked by 5' and 3' EcoRI restriction digestion sites, upstream of the cartilage oligomeric matrix protein pentamerization domain (COMP; residues 28-72 of SEQ ID NO: 12) followed by a C-terminal hexahistidine tag. An expression plasmid coding for COMP domain alone

(control) was constructed by excising the VISTA ECD region from the VISTA.COMP plasmid by EcoRI restriction digestion. All plasmids encoded a 5' Ig-kappa leader sequence for high protein secretion in mammalian cells. Recombinant proteins were expressed using the Expi-293TM transient expression system (Thermo Fisher Scientific). Human VISTA.COMP (hVISTA.COMP, SEQ ID NO: 24) was created as above but with the exception of DNA encoding for the hVISTA ECD (SEQ ID NO: 9) in place of the mouse VISTA ECD and the human COMP sequence (SEQ ID NO:11) in place of the mouse COMP domain. Secreted VISTA.Fc was purified from culture media using HiTrap Protein A HP columns (GE Healthcare), while the histidine tagged VISTA.COMP and COMP were purified using Ni-NTA resin (Qiagen) and desalted into PBS pH 7.4 using PD-10 columns (GE Healthcare). Proteins were verified for purity using SDS-PAGE, and protein concentration quantified by BCA assay (Pierce) or A280 measurements.

Animals

[00153] C57BL/6 mice used throughout this study were housed in a pathogen free environment at the Sunnybrook Research Institute Comparative Research (SRICR) facility while CD200R1^{-/-} mice were bred at the Toronto Research Institute Animal facility. All protocols were approved by the SRICR animal care committee, accredited by the Canadian Council of Animal Care.

Cell Culture

[00154] CD4⁺ T-cells were isolated from spleens of C57BL/6 mice using an EasySep Mouse CD4⁺ T-cell isolation kit (Stem Cell) and cultured in RPMI-1640 media supplemented with 10% FBS, penicillin (100U/mL), streptomycin (100 µg/mL) and 0.05mM 2-mercaptoethanol. The murine 2.10 T-cell clone was cultured in complete IMDM supplemented with IL-2 (3.5 µg/mL), lecithin (20 µg/mL), and BSA (0.5 mg/mL).

2.10 T-cell clone activation

[00155] 96-well microtiter plates were coated with anti-CD3 antibody (3µg/mL in PBS, clone 145-2C11, BioXcell) at 4^oC overnight. To monitor the effects of immobilized checkpoint ligands on 2.10 cell activation, the anti-CD3 coated wells were washed and coated with VISTA.COMP or other recombinant proteins for 1hr at 37^oC in PBS. Wells were then washed with PBS (3x) to remove residual unbound proteins. Murine 2.10 T-cells grown in culture were recovered, washed in IMDM (x3), and dispended into protein-coated wells (1x10⁴ cells/well). To measure proliferation, cells were pulsed for 6hr with 1µCi of [³H]-Thymidine after 18hr of culture and uptake quantified using a TopCount NXT scintillation counter (Perkin Elmer). To assay the sensitivity of 2.10 T-cells to soluble checkpoint ligands,

indicated recombinant proteins were diluted in culture media and added to anti-CD3 antibody-coated wells simultaneously with the 2.10 cells.

CD4+ T-cell Proliferation and Cytokine Secretion (mouse)

[00156] Isolated murine CD4+ T-cells were labelled with CFSE following the manufacturers protocol (Thermo Fisher Scientific) and stimulated in 96-well microtiter plates pre-coated with an anti-CD3 antibody in the presence of either murine VISTA.Fc, VISTA.COMP or COMP alone (coated or soluble). Cells were harvested 48 or 72hrs later and CFSE-dilution profiles quantified by flow cytometry (FACScalibur, Becton Dickinson). Culture media were harvested from stimulated CD4+ T-cells at 48 or 72hr and analysed by enzyme linked immunosorbent assay (ELISA, R&D System) to quantify VISTA.COMP-mediated inhibition of IL2 and IFN γ secretion.

Human T-cell activation and proliferation assays

[00157] Peripheral blood mononuclear cells (PBMCs) isolated from healthy donors (STEM CELL Technologies) were cultured with 5 μ g/mL ConA for 48 or 72 hours or immobilized anti-CD3 antibody (OKT3, 1 μ g/mL) in the presence of VISTA.Fc, VISTA.COMP, or COMP. In some cases, cells were labelled before culture with CFSE to trace proliferation. After culture cells were harvested, stained with the indicated antibody (anti-CD3, anti-CD4, anti-CD8, and/or anti-CD25) and analysed by flow cytometry.

Flow cytometry binding assays

[00158] Binding of VISTA.COMP, VISTA.Fc, or control proteins to T-cells was assessed using flow cytometry. Proteins were first biotinylated using EZ-Link Sulfo-NHS-LC-Biotin reagent (Thermo Scientific) as directed by the manufacturer. Upon completion of the reaction, the excess biotin was removed using a PD-10 (GE Healthcare) desalting column. To confirm equivalent levels of biotinylation of each protein, the quantity of biotin conjugated to each ligand was determined using HABA/Avidin reagent (Sigma). 2.10 T-cells were incubated with the indicated biotinylated protein (10 μ g/100 μ L) or VISTA.Fc for 0.5hr at 4°C in FACS staining buffer (PBS supplemented with 1% FBS and 0.09% NaN $_3$). After removal of non-bound proteins, cells were incubated with streptavidin-PE (1:300, BioLegend) or PE-anti-human IgG (1:100, BioLegend) in FACS staining buffer for 15 minutes and the PE-fluorescence signal analysed using a FACScalibur cell analyzer.

Allogeneic Mouse Mixed Leukocyte Culture Assay (allo-MLC)

[00159] VISTA.COMP or CD200Fc (positive control) were added to allogeneic murine mixed leukocyte cultures for 5 days and induction of cytotoxic T-lymphocytes (CTLs) assayed as previously described (Reference 39). Briefly, C57Bl/6 responder splenocytes

were incubated with an equal number of irradiated BALB/c stimulator cells in the presence of each recombinant protein at the indicated concentration. Induced CTLs were assayed by monitoring the release of ^{51}Cr from loaded P815 mastocytoma target cells over 5hrs (25:1 effector to target ratio).

Allogeneic skin graft transplant

[00160] The immunosuppressive effect of VISTA.COMP was tested *in-vivo* using a mouse skin allograft model as previously described (Reference 39). BALB/C mice received C57Bl/6 skin grafts (day 0) followed by treatment with VISTA.COMP (15 μg IV) once every 3 days for a total of 5 treatments in combination with low-dose rapamycin (0.5mg/kg, I.P. injections every 48 hrs). A blinded investigator monitored graft survival daily.

Concanavalin-A Induced Acute Hepatitis

[00161] The ability of VISTA.COMP to rescue mice from lethal acute inflammation was evaluated using the Con-A model of acute hepatitis. Male C57Bl/6 mice were treated I.P. with VISTA.COMP (200 μg) or PBS, two hours prior to I.V. injection of a lethal dose (15mg/kg) of Con-A (Sigma-Aldrich). A subset of animals were sacrificed after 3 hours to quantify serum IL-6 and TNF α levels by ELISA (R&D Systems) and the remaining animals were monitored for survival over the course of 24 hours.

Solid Phase Immunoprecipitation Assay

[00162] A solid phase immunoprecipitation assay was performed to assess the inhibitory effects of VISTA.COMP on TCR phospho-signalling cascades. 2×10^6 T-cells were exposed to plates coated with anti-CD3 antibody (with or without VISTA.COMP) for 15 minutes. Residual medium was removed and cells lysed in situ upon incubation with lysis buffer (50mM Tris pH 7.4, 150mM NaCl, 1% NP40, 5mM $\text{Na}_4\text{O}_7\text{P}_2$, 5mM NaF, 2mM Na_3VO_4 , and 1X Sigma Protease Inhibitor Cocktail) for 30 minutes at 4°C . Wells were vigorously washed 3x with lysis buffer, and adhered proteins eluted with 3.5% NH_4OH . The eluted proteins were lyophilized and resuspended in SDS-sample buffer, and total phosphorylated proteins visualized by western blot using an anti-phosphotyrosine antibody (clone 4G10; Sunnybrook Antibody Core Facility).

Statistics

[00163] Statistical analyses were performed using GraphPad Prism software (v6.0.2) using either Students T-test, or Mann Whitney U-tests where indicated. Graphs and visuals were created using GraphPad Prism software.

Example 2: VISTA.COMP Results**Dimeric VISTA suppresses T-cell proliferation only when immobilized**

[00164] The activation of immune checkpoint receptors on T-cells has, in some cases, been initiated through the binding of an IgV domain displayed by a protein ligand such as PD-L1 expressed on APCs and tumour cells to a complementary IgV domain of its cognate immune checkpoint receptor PD-1 on T-cells. Past studies have demonstrated that monomeric forms of these IgV domains involving PD1:PD-L1 and CD28:CD80/CD86 interact with each other with modest affinity, reflected by Kd values typically in the low micromolar (μM) range (References 40, 41). To activate checkpoint receptors on T-cells in-vitro, these immune checkpoint ligands have been expressed as oligomers, such as Fc fusion proteins, which have been immobilized on a surface. The immobilized presentation mimics avidity events taking place when such immune checkpoint domains are displayed on the surface of APCs and T-cells.

[00165] Consistent with previous reports (References 8, 9), a dimeric form of VISTA (VISTA-Fc), constructed by fusing the VISTA IgV domain with the Fc region from IgG1, suppressed the proliferation of anti-CD3 stimulated CD4+ T-cells only when it was immobilized on a culture dish (FIG. 1A). Soluble VISTA-Fc added to culture media during CD4+ T-cell stimulation had negligible impact on cell proliferation, suggesting that use of VISTA-Fc in-vivo to suppress T-cell activity may be limited due to its inability to fully agonize the putative VISTA-receptor.

[00166] Without being bound by theory, the lack of activity in soluble VISTA-Fc in-vitro may be caused by insufficient avidity towards its receptor and/or a lack of ability to cluster the VISTA-receptor on the cell surface.

[00167] A higher order VISTA oligomer was engineered in order to generate an agonist that may effectively suppress T-cell stimulation both in-vitro and in-vivo. A recombinant VISTA pentamer (VISTA.COMP; see SEQ ID NO: 14 of Appendix 1 for sequence) was constructed by genetically fusing the VISTA IgV domain to the COMP pentamerization domain. Recombinant VISTA.COMP was produced in a mammalian expression system, yielding a pentameric protein of ~250 kDa stabilized by intramolecular disulphide bonds within the COMP pentamerization domain (FIG. 1B).

VISTA.COMP suppresses T-cell activation and proliferation as a soluble ligand *in-vitro*

[00168] In contrast to VISTA-Fc, soluble VISTA.COMP substantially suppressed expansion and proliferation of isolated anti-CD3 stimulated CD4+ T-cells (Fig. 1C). The

recombinant COMP domain alone showed negligible effect on T-cell expansion and proliferation suggesting that VISTA.COMP activity is due to VISTA signalling, and not off-target events associated with the COMP domain. In addition, soluble VISTA.COMP significantly diminished ($p < 0.01$) the secretion of inflammatory cytokines IL-2 (FIG. 1D) and IFN γ (FIG. 1E) by stimulated CD4 $^{+}$ T-cells. The efficacy of VISTA.COMP suppression was inversely correlated with the strength of T-cell receptor (TCR) stimulation, as increased anti-CD3 stimulation led to increases in T-cell division in the presence of VISTA.COMP (FIG. 1F). In addition to its ability to suppress T-cell proliferation in response to a polyclonal stimulus, VISTA.COMP readily suppressed the induction of cytotoxic T-lymphocytes (CTLs) in a dose dependent manner in allogenic mixed-leukocyte cultures (FIG. 1G). These results demonstrate that VISTA.COMP is an effective agonist, capable of activating the VISTA-receptor on T-cells to regulate their activity. Unlike VISTA-Fc, VISTA.COMP does not require immobilization on a solid surface to exhibit agonistic activity.

hVISTA.COMP suppresses the activation and proliferation of human T-cells

[00169] A human version of VISTA.COMP (SEQ ID NO: 24) was constructed as described for mVISTA.COMP with replacement of the mouse VISTA ECD with that of hVISTA.COMP, and the replacement of mouse COMP pentamerization domain with that of human COMP. This protein – hVISTA.COMP (SEQ ID NO: 24) – was readily expressed by Expi293F cells and purified to homogeneity (FIG. 1H). Similar to what was previously observed in experiments with mouse T-cells, hVISTA.COMP, but not COMP, was found to readily suppress the proliferation of human T-cells isolated from adult PBMCs induced by ConA (FIG. 1I). Furthermore, hVISTA.COMP suppressed upregulation of the CD25 T-cell activation marker in human CD4 $^{+}$ and CD8 $^{+}$ T-cells undergoing anti-CD3 induced activation (FIG. 1J). Together these results indicate that similar to what was previously observed with mVISTA.COMP, human VISTA.COMP can induce VISTA-mediated immunoinhibitory signalling to suppress the activation of human T-cells.

VISTA.COMP binds to a clonal T-cell line and suppresses its activation

[00170] In addition to primary CD4 $^{+}$ T-cells, it was found that a CD4-negative murine IL-2 dependent T-cell clone (2.10) (Reference 42) was sensitive to VISTA inhibitory signalling, providing a controlled system to assay the effects of VISTA-receptor agonists. Consistent with what was observed in primary CD4 $^{+}$ T-cells, VISTA-Fc suppressed anti-CD3 induced proliferation only when immobilized on a solid surface, while VISTA.COMP suppressed activity when immobilized or when provided in a soluble form in culture media ($p < 0.01$) (FIG. 2A). Titration of soluble VISTA.COMP and VISTA-Fc demonstrated that VISTA.COMP suppressed anti-CD3-induced 2.10 cell proliferation at concentrations as low as 1 $\mu\text{g/mL}$

($p < 0.01$), whereas VISTA-Fc had no detectable activity at concentrations as high as 30 $\mu\text{g/mL}$ (FIG. 2B). In addition to suppressing proliferation, intracellular flow cytometry showed that soluble VISTA.COMP, but not VISTA-Fc, suppressed stimulated 2.10 cell IL-2 secretion within 4 hours of exposure ($p < 0.05$), suggesting an immediate and rapid effect of VISTA.COMP (FIG. 2D). VISTA.COMP suppressed the rapid phosphorylation of tyrosine residues within TCR-complex signalling proteins induced upon anti-CD3 stimulation of the 2.10 cells (FIG. 2E). Mechanistically, these results are consistent with the previous finding that exposing naïve CD4⁺ T-cells to immobilized VISTA-Fc, led to long-term suppression of T-cells upon transfer to anti-CD3 coated wells (in the absence of further VISTA-Fc), which suggests a role for VISTA signalling as an early regulator of T-cell activation⁹. Flow cytometry was then performed on the 2.10 cell line using VISTA-Fc, COMP, or VISTA.COMP, to determine if the inability of soluble VISTA-Fc to bind to the VISTA-receptor on T-cells contributes to the lack of suppressive activity. VISTA.COMP and COMP were labelled with an equivalent number of biotin groups, and cell-bound biotinylated proteins were detected with PE-streptavidin, while bound VISTA-Fc was detected with PE-anti-IgG. Both VISTA-Fc and VISTA.COMP were found to bind to naïve 2.10 T-cells while the baseline signal observed for COMP confirmed the absence of non-specific binding arising from the pentamerization domain alone (FIG. 2C). Unlike VISTA.COMP, the VISTA-Fc signal could be readily displaced by additional washing steps suggesting that its interaction with the putative VISTA receptor is of low affinity (FIGs. 2F-H). Altogether, these findings demonstrate that a soluble low-avidity VISTA ligand, such as VISTA-Fc, is not sufficient to activate immunoinhibitory signalling through this pathway *in-vitro*. Only the high-avidity VISTA.COMP ligand was capable of stimulating the VISTA-receptor.

VISTA.COMP suppresses immune responses *in-vivo*

[00171] In view of the data showing that VISTA.COMP suppresses T-cell activity *in-vitro* as a soluble ligand, it may be a useful agonist to suppress pro-inflammatory responses *in-vivo*. VISTA.COMP was first tested in a murine skin allograft model. BALB/C mice received non-histocompatible skin allografts (from C57Bl/6 donors) before receiving treatment with VISTA.COMP or a saline control combined with low-dose rapamycin (FIG. 3A). It was previously demonstrated that this dose of rapamycin has no effect on graft survival as a monotherapy (Reference 39). VISTA.COMP significantly prolonged the survival of skin allografts. Only 1/6 allografts were rejected in the VISTA.COMP treatment group at the last day of treatment (day 15) relative to 6/6 rejected allografts observed in the saline control group ($p < 0.05$, Mann-Whitney U-test) (FIG. 3B). The immunosuppressive effects of VISTA.COMP were also evaluated in an acute inflammatory hepatic model called Concanavalin-A (ConA) induced hepatitis. In this model, administration of ConA induces

acute liver inflammation mediated by a polyclonal activation of CD4⁺ T and NKT cells (Reference 43). This model was used to assess the suppressive activity of VISTA.COMP on T-cells *in-vivo*. Previous research in this model suggested that agonistic anti-VISTA antibodies directed towards VISTA on T-cells could rescue mice from lethal hepatic injury (Reference 10). However, the effect of treatment with VISTA-receptor agonist was unknown. It was found that prophylactic treatment of mice with VISTA.COMP rescued 3/4 of male C57Bl/6 mice from succumbing to a lethal dose of ConA (FIG. 3C). It was consistently found that serum levels of TNF α and IL-6 at a 3-hour time point, post-ConA injection, were significantly reduced upon VISTA.COMP treatment ($p < 0.05$) (FIG. 3D). The results from these two acute inflammatory disease models suggest that VISTA.COMP may serve as a strong agonist to suppress inflammatory responses *in-vivo*.

Influence of tag on VISTA.COMP activity

[00172] Proliferation of anti-CD3 stimulated CFSE-labelled splenic CD4⁺ T-cells in the presence of coated or soluble VISTA constructs (FIG. 4A). Soluble VISTA.Fc had no suppressive effects on the CD4⁺ T-cells, while VISTA.COMP exhibited immunosuppressive activity on the CD4⁺ T-cells without requiring immobilization. Further, histidine tagged VISTA.COMP (VISTA.COMP.his; SEQ ID NO: 14 in Appendix 1) exhibited increased activity relative to strep II tagged VISTA.COMP (VISTA.COMP.SS; SEQ ID NO: 60), suggesting an influence of tag on protein stability. Using SPR binding assays, a commercial anti-VISTA antibody (clone 730802) RnD systems) recognized VISTA.COMP.his but not VISTA.COMP.SS (FIG. 4B) suggesting a fundamental change in epitope exposure (+ indicates detectable binding by SPR, - indicated complete absence of binding).

VISTA.COMP is a high-avidity checkpoint receptor agonist

[00173] The data provided herein suggest that VISTA.COMP is a high-avidity checkpoint receptor agonist capable of suppressing T-cell activities *in-vitro* and capable of suppressing inflammatory responses *in-vivo*. Comparisons between immobilized and soluble VISTA-Fc and VISTA.COMP show that activity as a VISTA-receptor agonists is dependent on the level of oligomerization, the higher-avidity multimer created using the COMP pentamerization domain being required for activity in solution (i.e., in the absence of immobilization to a substrate). The inventors have found the COMP domain to be a useful scaffold for expressing stable VISTA pentamers. The data provided herein, combined with the observation of exacerbated autoimmune diseases observed upon genetic deletion of VISTA in mice, suggest a potential utility of targeting the VISTA-mediated immunosuppression pathway to clinically suppress undesired immune responses.

Example 3: ICOS-L.COMP Methods**Recombinant ICOS-L.COMP expression and purification**

[00174] A dsDNA construct was synthesized (GeneArt; Thermo Fisher Scientific) containing dsDNA encoding the human ICOS-L extracellular domain (ECD)(SEQ ID NO: 49) upstream of dsDNA encoding the human COMP pentamerization domain (SEQ ID NO: 11) with a C-terminal histidine tag and inserted in the pcDNA3.4 expression plasmid (GeneArt; Thermo Fisher Scientific). The sequence also contained the Ig-kappa leader sequence at the 5' end of the ICOS-L ECD, and the nucleotide sequences were codon optimized to allow high yield secretion from human derived cell lines (ICOS-L.COMP; see SEQ ID NO: 57 of Appendix 1 for sequence). The ICOS-L.COMP encoding plasmid was transfected into Expi293F cells following manufacturers recommendations (GeneArt; Thermo Fisher Scientific) and a stable cell line secreting ICOS-L.COMP was selected by exposing the transfected cell to geneticin (GeneArt; Thermo Fisher Scientific) for two-weeks. Secreted histidine-tagged ICOS-L.COMP was purified from cell-culture supernatants using HisTrap HP columns (GE Healthcare). Subsequent purification, protein samples were desalted into PBS, pH 7.4, using PD10 columns (GE Healthcare). Proteins were verified for purity using SDS-PAGE, and protein concentration quantified by BCA assay (Pierce) or A280 measurements.

Animals

[00175] C57BL/6 mice used throughout this study were housed in a pathogen free environment at the Sunnybrook Research Institute Comparative Research (SRICR) facility. All protocols were approved by the SRICR animal care committee, accredited by the Canadian Council of Animal Care.

Human T-cell proliferation and activation

[00176] The ability of ICOS-L.COMP or ICOSL-Fc (R&D Systems) to co-stimulate human T-cells in vitro as a soluble ligand was assayed. Human T-cells were isolated from Ficoll-Paque separated human cord blood cells or adult PBMCs using the EasySep Human T-cell Isolation Kit (STEMCELL Technologies). Isolated T-cells were labelled with CFSE following manufacturers protocol (Thermofisher) and stimulated in 96-well plates coated with an anti-CD3 antibody (clone OKT3, BioXcell). Cells in each well were cultured in RPMI-1640 media supplemented with 10% FBS, penicillin (100U/mL), streptomycin (100 µg/mL) and 0.05mM 2-mercaptoethanol. Selected wells were incubated with soluble COMP or ICOS-L.COMP at titrated concentrations. Cells were harvested 48-72 hours later, stained with the appropriate antibody (anti-CD4, anti-CD8, and/or anti-CD25) and analysed by flow cytometry (FACScalibur, Becton Dickinson) for proliferation (CFSE) and upregulation of activation

markers (i.e., CD25). Cell culture supernatants from these wells were also collected for cytokine analysis at 72hr and secretion of IFN γ , TNF α , IL10, IL2 and IL6 quantified using the human LEGENDplex Th1 inflammation panel (Biolegend).

ICOS-L.COMP binding to hICOS, mICOS, and CD28

[00177] Direct binding of ICOS-L.COMP to hICOS, mICOS, and hCD28 was evaluated using Biacore T-200 surface plasmon resonance (SPR) experiments. hICOS-Fc, mICOS-Fc, and hCD28-Fc (all from R&D Systems) were affinity captured (350-400RU) by Protein A (Sigma Aldrich) previously amine coupled to a CM5 chip (GE Healthcare). hICOS-L.COMP was injected over each affinity captured protein at a concentration of 25nM in HBS-EP running buffer (GE Healthcare).

ICOS-L.COMP binding kinetics

[00178] The kinetics of hICOSL-COMP, hICOSL-Fc, and COMP binding to immobilized hICOS-Fc was determined by SPR single cycle kinetic analysis using a Biacore T200. Briefly, titrated concentrations of ICOS-L.COMP, ICOSL-Fc (R&D Systems), or COMP (negative control) were injected over hICOS-Fc (R&D Systems) previously immobilized on a CM5 sensor chip (GE Healthcare). The derived sensorgrams were fit with a 1:1 binding model to determine on-rate (k_a), off-rate (k_d) and the dissociation constant (K_D).

ICOS-L.COMP binding to human T-cells

[00179] The ability of ICOSL-COMP to bind to human T-cells was demonstrated by flow cytometry. ICOS-L.COMP was derivatized with FITC following manufacturer's directions (ThermoFisher). 1×10^5 CD3+ T-cells previously isolated from human donor PBMCs (STEMCELL technologies human CD3+ Isolation Kit) was incubated with 100nM ICOS-L.COMP.FITC and PE-Cy7-anti-CD4 (Biolegend) for 20 minutes in FACS buffer (PBS + 2%FBS + 0.09% NaN₃). Cells were subsequently washed and analysed by flow cytometry using a BD LSR cytometer. DAPI was used to exclude dead cells.

ICOS-L.COMP competition experiments (human)

[00180] The ability of hICOS-L.COMP to compete with hICOS-Fc for binding to ICOS on 1-day anti-CD3/CD28 stimulated human CD3+ T-cells was evaluated by flow cytometry. Briefly, 200nM ICOS-L.COMP.FITC or an equal volume of PBS (no ICOS-L.COMP) was pre-incubated with stimulated CD3+ T-cells for 15 minutes on ice prior to addition of 100nM hICOS-L.Fc. Cells were washed and incubated with a PE labelled anti-human IgG-Fc secondary antibody (Biolegend), and analysed by flow cytometry using a BD LSR cytometer.

ICOS-L competition experiments (mouse)

[00181] The ability of hICOS-L.COMP to compete with mICOS-Ig for binding to ICOS on primary murine CD4⁺ was evaluated by flow cytometry. Splenic murine CD4⁺ T-cells were isolated using the EasySep Mouse CD4⁺ T-cell isolation kit (STEMCELL Technologies) and activated for 48 hours by exposure to immobilized anti-CD3 antibody (clone 145-2C11, BioXcell) to upregulate ICOS expression. Activated T-cells were incubated with mICOS-Ig or mICOS-Ig combined with ICOS-L.COMP, and binding of mICOS-Ig detected using a PE conjugated anti-human IgG-Fc antibody (BioLegend).

MC38 colon carcinoma mouse model

[00182] The ability of ICOS-L.COMP to synergize with anti-PD-1 checkpoint blockade to restore anti-tumor immune responses and slow the progression of established tumors was demonstrated using the MC38 colon carcinoma model. Male C57BL/6 mice were injected with 2x10⁵ MC38 tumor cells and tumors allowed to reach a size of 50-150mm³ prior to treatment over the course of 7-10 days. Animals were subsequently injected with either PBS, anti-PD-1 (200µg, clone RMPI-14), ICOS-L.COMP (100µg) or anti-PD-1 combined with ICOS-L.COMP every 2-3 days for five injections. Tumor size was measured every other day using calipers and calculated using the formula: large diameter x small diameter² x π / 6. In some cases tumors were resected at day 10-12 post treatment initiation, enzymatically dissociated to a single cell suspension, and stained with anti-CD45, anti-CD4, anti-CD8, anti-FOXP3 and anti-ICOS to profile TIL populations after treatment.

Example 4: ICOS-L.COMP Results

Rationale and design of a pentameric ICOS-L fusion protein

[00183] T-cells require two-signals to achieve activation in-vitro and in-vivo, with the first signal being delivered by the T-cell receptor (TCR) upon recognition of antigen displayed on the major histocompatible complex (MHC). Secondary co-stimulatory signals are delivered by a number of ligand:receptor interactions such as B7-1/2:CD28 to increase T-cell activity. ICOS, a member of the B7/CD28 family, is a co-stimulatory receptor which is upregulated by T-cells upon activation. Binding of ICOS by its ligand ICOS-L, which is expressed on antigen presenting cells (APC), leads to increased T-cell proliferation and cytokine production. Prior studies have demonstrated increased expression of ICOS on T-cells in cancer patients treated with anti-CTLA-4 (ipilimumab), with this upregulation being correlated with improved clinical outcome. Pre-clinical experiments have established a therapeutic utility in agonizing ICOS signalling to promote beneficial anti-tumor immune responses. Specifically, it was shown that agonizing ICOS using a whole-cell vaccine (ICOS-L expression on B16 melanoma tumor cells) in combination with anti-CTLA-4 could lead to a delay in tumor growth in the B16 melanoma model. Collectively these results confirm a role for an ICOS

agonist as a synergistic therapeutic to established checkpoint blocking therapeutics (anti-PD-1 and anti-CTLA-4 monoclonal antibodies).

[00184] To this end, it was hypothesized that a soluble ICOS agonist could be derived by pentamerization of the ICOS-L extracellular binding domain. Due to an increased avidity and clustering, it was hypothesized that this pentameric version of the natural ligand for ICOS (ICOS-L) may agonize ICOS signalling to a greater extent than would agonistic ICOS monoclonal antibodies or a dimeric version of the ICOS-L ECD (i.e., ICOS-Fc).

[00185] A pentameric ICOS-L construct was created by genetic fusion of the ICOS-L ECD (IgV+IgC domain) to the COMP pentamerization domain (ICOS-L.COMP; see SEQ ID NO: 57 of Appendix 1 for sequence). ICOS-L.COMP was expressed in a mammalian cell expression system, yielding stable homopentamers of MW~300kDa under non-reducing conditions (FIG. 8B).

ICOSL.COMP binds to human and mouse ICOS but not CD28

[00186] hICOS-L.COMP was characterized for its binding to ICOS and the closely related family member CD28 by SPR. hICOSL-COMP at 25nM readily bound both mouse and human ICOS as expected (FIG. 9A). Very modest to negligible binding to hCD28 was observed exemplifying the specificity of this interaction.

ICOS-L.COMP binds to ICOS with superior affinity/avidity than ICOSL-Fc

[00187] Surface plasmon resonance (SPR) assays were used to characterize the binding affinity of hICOS-L.COMP and ICOSL-Fc to hICOS. hICOS-L.COMP bound with an apparent KD of 0.9 nM, a value approximately 3-fold stronger than hICOSL-Fc (2.9nM). COMP did not show binding to ICOS demonstrating that this interaction is due to specific ICOSL-ICOS binding (FIG. 9B).

hICOS-L.COMP binds directly to human T-cells and competes with ICOSL-Fc

[00188] hICOSL-COMP was also characterized for its ability to bind to ICOS-expressing human CD3+ T-cells. For this, hICOSL-COMP was derivatized with FITC, yielding approximately 13-15 FITC/molecule. hICOSL.COMP.FITC was found to readily bind CD3+CD4+ and CD3+CD4- T-cells isolated from human PBMCs (FIG. 9C). Additionally, the binding of ICOS-L.COMP completely inhibited binding of ICOSL-Fc to stimulated human T-cells, confirming that ICOS-L.COMP and ICOSL-Fc compete for binding to cellular expressed ICOS (FIG. 9D).

ICOS-L competition experiments (mouse)

[00189] The ability of hICOS-L.COMP to outcompete mICOS-Ig for binding to ICOS displayed on primary murine CD4⁺ was evaluated by flow cytometry. The pentameric hICOS-L.COMP sufficiently outcompetes mICOS-L-Ig for binding to ICOS expressed by activated T-cells (FIG. 9E).

ICOS-L.COMP co-stimulates human T-cells

[00190] The function of ICOS-L.COMP in agonizing ICOS signalling to co-stimulate human T-cells was established. CFSE labelled CD3⁺ T-cells isolated from fresh human cord blood were stimulated in vitro with anti-CD3 antibody, and ICOS-L.COMP or COMP (negative control) added soluble to the culture media. Cell expansion and proliferation was tracked after 72 hours by FACS, demonstrating that ICOS-L.COMP, but not COMP, profoundly stimulates the proliferation of CD4⁺ and CD8⁺ T-cells (FIG. 10A). Additionally, ICOS-L.COMP, induced upregulation of the T-cell activation marker CD25 (FIG. 10B). In stark contrast to hICOSL-COMP, ICOSL-Fc as a soluble ligand was not capable of inducing the expansion and proliferation of CD3⁺CD4⁺ T-cells (FIG. 10C). Lastly, ICOSL-COMP did not stimulate T-cells in the absence of anti-CD3 induced T-cell receptor signalling confirming the function of ICOSL-COMP as a co-stimulatory ligand (FIG. 10D). Similar results were also seen in the co-stimulation of T-cells isolated from adult human PBMCs (FIG. 10E). Furthermore, soluble ICOSL-COMP co-stimulation, but not ICOSL-Fc, led to substantial increases in the secretion of Th1 cytokines IFN γ , TNF α , and IL10, and modest but significant increases in IL2 and IL6. (FIG. 10F). Collectively these experiments demonstrate that soluble ICOS-L.COMP readily agonizes the ICOS signalling pathway to stimulate human T-cell proliferation and activation.

ICOS-L.COMP synergizes with checkpoint blockade to promote protective anti-tumor immunity in mice

[00191] To determine the anti-tumor efficacy of ICOS-L.COMP in combination with checkpoint blockade, the MC38 colon carcinoma model was used. Here, immune competent C57Bl/6 mice with injected subcutaneously with MC38 cells and tumors established to a volume of 50-150mm³ prior to treatment with PBS (control), anti-PD-1 monotherapy, ICOS-L.COMP monotherapy, or ICOS-L.COMP combined with anti-PD-1 (combination) (FIG. 11A). Combination treatment with ICOS-L.COMP and anti-PD-1 led to a significantly delayed tumor growth relative to anti-PD-1 monotherapy (**P<0.01) (FIG. 11B). Individual tracing of each animal's tumors shows complete responses were observed in 3/12 animals in the combination group at the time of last treatment (FIG. 11C), with zero instances of complete regression in any other group. Animals in the ICOS-L.COMP monotherapy group did not exhibit improved outcome compared to PBS controls suggesting the need for checkpoint

blockade combination in this model (FIG. 12A). Supporting a role for the combination approach, tumor infiltrating CD4⁺ and CD8⁺ T-cells had significantly higher ICOS expression and an overall increase in ICOS⁺ cells within the tumor (FIG. 12B). Together this suggests that the efficacy of checkpoint blocking antibodies which upregulate ICOS expression and increase the abundance of TILs can be potentiated by a combination treatment with a soluble ICOS agonist. Supportingly, combined treatment with anti-PD-1 and ICOS-L.COMP led to modest but significant increases in CD4⁺FOXP3⁻ (CD4 effector) cells within the TIL compartment (CD45⁺) compared to anti-PD-1 monotherapy alone (*P<0.05) (FIG. 12C). There were no changes in the abundance of Treg cells (CD4⁺FOXP3⁺) (FIG. 12D).

[00192] Collectively the data show that an ICOS-L ECD pentamer (ICOS-L.COMP) can readily bind to ICOS with high-avidity to co-stimulate human T-cells. Importantly, this co-stimulation in-vivo can lead to an increase in anti-tumor activity when combined with checkpoint blockade (anti-PD-1 antibody) leading to a reduced tumor burden.

Example 5: PD-L1.COMP Methods

Design of a PD-L1 pentamer (PD-L1.COMP) expression vector

[00193] A codon optimized dsDNA construct was synthesized (IDT) encoding the mouse PD-L1 ECD bearing EcoRI (5') and KpnI (3') restriction sites. This construct was digested, and ligated into a EcoRI/KpnI double digested plasmid (COMP.HIS8-pcDNA3.4) such that the PD-L1 ECD (SEQ ID NO:42) was located downstream an Ig-kappa leader sequence and upstream the COMP pentamerization domain (SEQ ID NO: 12) (PD-L1.COMP; see SEQ ID NO: 46 of Appendix 1 for sequence).

Expression and purification of chimeric PD-L1.COMP

[00194] The PD-L1.COMP encoding plasmid was transfected into Expi293F cells following manufacturers recommendations (GeneArt; Thermo Fisher Scientific) and a stable cell line secreting PD-L1.COMP selected by exposing the transfected cell to geneticin (GeneArt; Thermo Fisher Scientific) for two weeks. Secreted histidine-tagged PD-L1.COMP was purified from cell-culture supernatants using HisTrap HP columns (GE Healthcare). Subsequent to purification, protein samples were desalted into PBS, pH 7.4, using PD10 columns (GE Healthcare). Proteins were verified for purity using SDS-PAGE, and protein concentration quantified by BCA assay (Pierce) or A280 measurements.

T-cell line activation and proliferation assays

[00195] The murine 2.10 T-cell clone was cultured in complete IMDM supplemented with IL-2 (3.5 µg/mL), lecithin (20 µg/mL), and BSA (0.5 mg/mL). For activation assays, cells were harvested, washed three times and seeded on an anti-CD3 (3ug/mL, BioXcell) coated

96-well plate at 2×10^4 cells/well. PD-L1.COMP and COMP (negative control) were either coated in the anti-CD3 coated wells in PBS pH7.4 for 1 hour at 37°C or added to the wells soluble with the 2.10 cells, at 10ug/mL construct concentration. Cells were cultured for 18 hours then pulsed with 1uCi [3H]-thymidine and cultured for another 6 hours. [3H]-thymidine uptake and proliferation quantified using TopCount NXT scintillation counter (Perkin Elmer).

Binding to a PD-1 expression T-cell clone

[00196] The binding of PD-L1.COMP to cell expressed PD-1 was investigating by flow cytometry using the 2.10 T-cell line. PD-L1.COMP was biotinylated using the EX-Link Sulfo-NHS-LC-Biotin reagent following manufacturer's directions (Thermo Scientific) and desalted into PBS using a PD10 column to remove excess biotin reagent. Resting or anti-CD3 activated 2.10 cells were incubated with biotinylated COMP or PD-L1.COMP for 30 minutes at 4C. Cells were subsequently washed in PBS and stained with Streptavidin-PE (1:100, BioLegend) for 30 minutes at 4C. The cells were washed with PBS, resuspended in PBS + DAPI (for live/dead cell exclusion), and read using a FACScalibur cell analyzer (Becton Dickinson).

Primary CD4+ T-cell line activation and proliferation

[00197] CD4+ T-cells were isolated from murine splenocytes using the mouse CD4+ T-cell isolation kit (STEM CELL Technologies). CD4+ T cells were CFSE labeled using manufacturer protocol (Thermo Fischer Scientific), and seeded on an anti-CD3 (3ug/mL) coated 96-well plate, with PD-L1.COMP, hB7-H4.COMP, mVISTA.COMP or COMP added soluble in culture media at 10µg/mL. Cells were harvested after 72 hours and CFSE profiles analysed by FACS (FACScalibur, Becton Dickinson). In some cases, culture media from the CFSE labeled cells were collected at 48 and 72 hour time points, and IL-2 and IFN γ secretion quantified by ELISA (R&D Systems).

Example 6: PD-L1.COMP results

[00198] Stimulation of negative checkpoint receptors occurs through the binding of an IgV domain displayed by a protein ligand, such as PD-L1, expressed on APCs and tumour cells to a complementary IgV domain of its cognate immune checkpoint receptor, such as PD-1, on T-cells. Past studies have demonstrated that monomeric forms of these IgV domains involving PD1:PD-L1 and CD28:CD80/CD86 interact with each other with modest affinity, reflected by Kd values typically in the low micromolar (μ M) range (References 40, 41). To activate checkpoint receptors on T-cells in-vitro, these immune checkpoint ligands have been expressed as dimers, such as Fc fusion proteins, which have been immobilized on a surface. The immobilized presentation mimics avidity events taking place when such immune checkpoint domains are displayed on the surface of APCs and T-cells. Previous

reports have shown that PD-L1-Fc requires immobilization on plates or beads to successfully agonize PD-1 immunoinhibitory signalling suggesting that use of PD-L1-Fc in-vivo to suppress T-cell activity may be limited due to its inability to fully agonize PD-1.

[00199] Without being bound by theory, the lack of activity in soluble PD-L1 in-vitro may be caused by insufficient avidity towards its receptor and/or a lack of ability to cluster PD-1. This hypothesis is further supported by the finding that a soluble VISTA IgV pentamer (VISTA.COMP) could readily suppress T-cell proliferation in-vitro while the dimeric VISTA-Fc could not.

Design, expression, and purification of a PD-L1 homopentamer: PD-L1.COMP

[00200] A higher order PD-L1 ECD multimer was engineered in order to generate a PD-1 agonist that may effectively suppress T-cell stimulation both in-vitro and in-vivo. A recombinant PD-L1 pentamer (PD-L1.COMP; see SEQ ID No: 46 in Appendix 1 for sequence) was constructed by genetically fusing the mouse PD-L1 ECD domain to the COMP pentamerization domain. Recombinant PD-L1.COMP was produced in a mammalian expression system, yielding a pentameric protein of ~250-300 kDa stabilized by intramolecular disulphide bonds within the COMP pentamerization domain (FIG. 5A).

PD-L1.COMP binds to PD-1 expressed by a T-cell line

[00201] Flow cytometry was performed on the 2.10 T-cell line using biotinylated PD-L1.COMP or COMP to establish that PD-L1.COMP binds to PD-1 expressed in a cell context. PD-L1.COMP and COMP were labelled with an equivalent number of biotin groups and used to stain naïve or anti-CD3 activated 2.10 cells. PD-L1.COMP, but not COMP readily bound to naïve 2.10 T-cells, with the amount of binding increased throughout T-cell activation, consistent with the established kinetics of PD-1 upregulation during T-cell activation (FIG. 13).

Primary CD4+ T-cell line activation and proliferation

[00202] In contrast to PD-L1-Fc which requires immobilization to agonize PD-1 and suppress T-cell activity, soluble PD-L1.COMP completely suppressed the expansion and proliferation of CFSE labelled primary murine CD4+ T-cells undergoing anti-CD3 antibody mediated stimulation. Importantly, recombinant COMP domain alone did not significantly inhibit T-cell proliferation confirming that the immunosuppressive effects seen with PD-L1.COMP are not off-target events due to the COMP pentamerization domain or histidine tag (FIG. 6A).

Example 7: B7-H4.COMP Methods

Design of a pentameric B7-H4 construct

[00203] The codon optimized gene fragment encoding human B7-H4 fused to the COMP pentamerization domain was synthesized (GeneArt, Thermo Fisher Scientific) and cloned into the pcDNA3.4 expression plasmid with a 5' Ig-Kappa leader sequence. The final construct consists of dsDNA encoding the human B7-H4 ECD (SEQ ID NO: 25) followed by a spacer sequence, the COMP pentamerization domain (SEQ ID NO: 11) followed by another spacer sequence and a HIS8 tag (B7-H4.COMP; see SEQ ID NO: 30 of Appendix 1 for sequence).

Expression and purification of pentameric B7-H4.COMP

[00204] hB7-H4.COMP was expressed using the Expi293 transient mammalian expression system using manufacturer's protocols (Thermo Fisher Scientific). Secreted hB7-H4.COMP was dialysed against PBS and purified by Ni-NTA purification using HisTrap HP columns. The protein was desalted into PBS pH 7.4 using a PD-10 column (GE Healthcare) and purity verified by SDS-PAGE and concentration determined using A280 measurements.

2.10 T-cell activation assay

[00205] The clonal IL-2 dependent 2.10 T-cell was cultured in complete IMDM supplemented with IL-2 (3.5 µg/mL), lecithin (20 µg/mL), and BSA (0.5 mg/mL). Cells were harvested, washed three times, resuspended in complete IMDM without IL-2 and lecithin and seeded on an anti-CD3 (3ug/mL, BioXcell) coated 96-well plate at 2×10^4 cells/well. hB7-H4.COMP, Vista.COMP (positive control) and COMP (negative control) were either coated in the anti-CD3 coated wells in PBS for 1 hour at 37°C and well subsequently washed, or added to directly to the wells soluble with the 2.10 cells. Cells were cultured for 18 hours then pulsed with 1uCi [3H]-thymidine and cultured for another 6 hours. [3H]-thymidine uptake and proliferation was quantified using TopCount NXT scintillation counter (Perkin Elmer).

Primary CD4+ T-cell line activation and proliferation

[00206] CD4+ T-cells were isolated from murine splenocytes using the mouse CD4+ T-cell isolation kit (STEM CELL Technologies). CD4+ T cells were CFSE labeled using manufacturer protocol (Thermo Fischer Scientific), and seeded on an anti-CD3 (3ug/mL) coated 96-well plate, with PD-L1.COMP, hB7-H4.COMP, mVISTA.COMP or COMP added soluble in culture media at 10µg/mL. Cells were harvested after 72 hours and CFSE profiles analysed by FACS (FACSCalibur, Becton Dickinson). In some cases, culture media from the CFSE labeled cells were collected at 48 and 72 hour time points, and IL-2 and IFN γ secretion quantified by ELISA (R&D Systems).

Binding to a T-cell clone

[00207] The binding of B7-H4.COMP to its putative receptor (B7-H4R) expressed on T-cells was investigated by flow cytometry using the 2.10 T-cell line. B7-H4.COMP was biotinylated using the EX-Link Sulfo-NHS-LC-Biotin reagent following manufacturer's directions (Thermo Scientific) and desalted into PBS using a PD10 column to remove excess biotin reagent. Resting or anti-CD3 activated 2.10 cells were incubated with biotinylated COMP or B7-H4.COMP for 30 minutes at 4°C. Cells were subsequently washed in PBS and stained with Streptavidin-PE (1:100, BioLegend) for 30 minutes at 4°C. The cells were washed with PBS, resuspended in PBS + DAPI (for live/dead cell exclusion), and read using a FACScalibur cell analyzer (Becton Dickinson).

Example 8: B7-H4.COMP Results

[00208] Previous reports have shown that B7-H4-Fc requires immobilization on plates or beads to successfully agonise immunoinhibitory signalling in T-cells suggesting that use of B7-H4-Fc in-vivo to suppress T-cell activity may be limited due to its inability to fully agonize the putative receptor in the absence of FcR-mediated cross-linking.

[00209] Without being bound by theory, the lack of activity of soluble B7-H4-Fc in-vitro may be caused by insufficient avidity towards its receptor and/or a lack of ability to cluster the putative receptor. This hypothesis is further supported by the finding that a soluble VISTA IgV pentamer (VISTA.COMP) could readily suppress T-cell proliferation in-vitro while the dimeric VISTA-Fc could not.

Design and expression of a B7-H4 pentamer

[00210] To address the issue of insufficient avidity or lack of clustering, a pentameric version of human B7-H4 was generated, by fusing the B7-H4 ECD with the COMP pentamerization domain (B7-H4.COMP; see SEQ ID NO: 30 of Appendix 1 for sequence). B7-H4.COMP was readily produced in mammalian cells and purified to homogeneity as stable pentamers at the MW of 250-300kDa (FIG. 5A).

hB7-H4.COMP binds to a clonal T-cell line and suppresses its activation

[00211] Flow cytometry was performed on the 2.10 T-cell line using biotinylated B7-H4.COMP or COMP to establish that B7-H4.COMP binds to the putative B7-H4R expressed on T-cells. B7-H4.COMP and COMP were labelled with an equivalent number of biotin groups and used to stain naïve or anti-CD3 activated 2.10 cells. B7-H4.COMP, but not COMP readily bound to naïve and activated 2.10 T-cells (FIG. 13).

[00212] The IL-2 dependent 2.10 T-cell line was used as a reporter system to assay if B7-H4.COMP could suppress T-cell activation. Consistent with previous observations using pentameric VISTA.COMP, B7-H4.COMP suppressed anti-CD3 induced proliferation whether

present as an immobilized ligand or provided in a soluble form in culture media ($p < 0.01$) (FIG. 14). The COMP pentamerization domain did not substantially alter proliferation when added as a soluble ligand, confirming on-target effects of VISTA.COMP and B7-H4.COMP.

B7-H4.COMP inhibits primary CD4⁺ T-cell line activation and proliferation

[00213] In contrast to B7-H4-Fc which requires immobilization to agonize B7-H4R and suppress T-cell activity, soluble B7-H4.COMP completely suppressed the expansion and proliferation of CFSE labelled primary murine CD4⁺ T-cells undergoing anti-CD3 antibody mediated stimulation (FIG. 15). Importantly, recombinant COMP domain alone did not significantly inhibit T-cell proliferation confirming that the immunosuppressive effects seen with B7-H4.COMP are not off-target events due to the COMP pentamerization domain or histidine tag. In addition, soluble B7-H4.COMP significantly diminished ($p < 0.01$) the secretion of the inflammatory cytokine, IL-2, by stimulated CD4⁺ T-cells (FIG. 16).

Pentamerization of checkpoint ligands as a strategy to design high-avidity checkpoint receptor agonists

[00214] The data provided herein indicate that pentamerization of three checkpoint ligands, namely PD-L1, B7-H4, and VISTA, can be used to design high-avidity checkpoint receptor agonist capable of suppressing T-cell activities in-vitro and capable of suppressing inflammatory responses in-vivo. Comparisons between immobilized and soluble VISTA-Fc and VISTA.COMP show that activity as checkpoint-receptor agonists is dependent on the level of oligomerization, with the higher-avidity multimer created using the COMP pentamerization domain being required for activity in solution (i.e., in the absence of immobilization to a substrate). The inventors have found the COMP domain to be a useful scaffold for expressing stable pentamers upon fusion of the ECD from checkpoint ligands. The data provided herein, combined with the observation of exacerbated autoimmune diseases observed upon genetic deletion of checkpoint ligands and receptors in mice, suggest a potential utility in agonizing these checkpoint receptors with pentameric agonists to clinically suppress undesired immune responses.

[00215] Although the invention has been described with reference to certain specific embodiments, various modifications thereof will be apparent to those skilled in the art without departing from the purpose and scope of the invention as outlined in the claims appended hereto. Any examples provided herein are included solely for the purpose of illustrating the invention and are not intended to limit the invention in any way. Any drawings provided herein are solely for the purpose of illustrating various aspects of the invention and are not intended to be drawn to scale or to limit the invention in any way. The disclosures of all prior art recited herein are incorporated herein by reference as if set forth in their entirety.

APPENDIX 1: SEQUENCES**VISTA extracellular domain cDNA sequence (human) (SEQ ID NO: 1)**

ttc
aaggctgccca cgccgtattc cctgtatgtc tgtcccagg ggcagaacgt caccctcacc
tgcaggctct tgggccctgt ggacaaaggg cagcatgtga cttctacaa gacgtggtag
cgcagctcga ggggcgaggt gcagacctgc tcagagcgcc ggcccatccg caacctcag
ttccaggacc ttcacctgca ccatggaggc caccaggctg ccaacaccag ccacgacctg
gctcagcgcc acgggctgga gtcggcctcc gaccaccatg gcaacttctc catcaccatg
cgcaacctga cctgtctgga tagcggcctc tactgtgccc tgggtgtgga gatcaggcac
caccactcgg agcacagggt ccatggtgcc atggagctgc aggtgcagac aggcaaagat
gcaccatcca actgtgtggt gtacccatcc tcctcccagg atagtgtgaaa catcacggct

VISTA extracellular domain cDNA sequence (mouse) (SEQ ID NO: 2)

ttcaaggta cactccata
ttctcttat gtgtgtccg agggacagaa tgccaccctc acctgcagga ttctgggccc
cgtgtccaaa gggcacgatg tgaccatcta caagacgtgg tactcagct cagaggcga
ggtccagatg tgcaagaac accggcccat acgcaacttc acattgcagc acctcagca
ccacggaagc cactgaaag ccaacgccag ccatgaccag cccagaagc atgggtaga
gtagcttct gaccaccag gtaacttctc tatcaccctg cgcaatgtga cccaaggga
cagcggcctc tactgtgtc tagtgataga attaaaaaac caccaccag aacaacggtt
ctacgggtcc atggagctac aggtacaggc aggcaaaggc tcgggggtcca catgcatggc
gtctaattag caggacagt acagcatcac ggct

COMP pentamerization domain cDNA sequence (human) (SEQ ID NO: 3)

gacctgggcc cgcagatgct tcgggaactg caggaaacca acgcggcgct gcaggacgtg
cgggagctgc tgcggcagca ggtcaggag atcacgttc tgaaaaacac ggtgatggag
tgtgacgct gcggg

COMP pentamerization domain cDNA sequence (mouse) (SEQ ID NO: 4)

gacct ggccccacag
atgtgctgag aactcagga gactaatgct gcgctgaag acgtgagaga gctgttgca
cagcaggta aggagatcac ctctgaag aatacggta tggaatgtga tgctgcga

VISTA extracellular domain mRNA sequence (human) (SEQ ID NO: 5)

uuc
aaggucgcca cgccguauuc ccuguauguc ugucccgagg ggcagaacgu caccucacc
ugcaggcucu ugggcccuu ggacaaaggg cacgauguga ccuucuacaa gacgugguac
cgcagcucga ggggcgaggu gcagaccugc ucagagcgcc ggcccauccg caaccucag
uuccaggacc uucaccugca ccauggaggc caccaggcug ccaacaccag ccacgaccug
gcucagcgcc acgggcugga gucggccucc gaccaccaug gcaacuucuc caucaccaug
cgcaaccuga ccugcugga uagcggccuc uacugcugcc ugguggugga gaucaggcac
caccacucgg agcacagggu ccauggugcc auggagcugc aggugcagac aggcaaagau
gcaccaucca acuguguggu guaccaucc uccucccagg auagugaaaa caucacggcu

VISTA extracellular domain mRNA sequence (mouse) (SEQ ID NO: 6)

uucaaggua ccacuccaua
uucucucuau guguguccc agggacagaa ugccaccuc accugcagga uucugggcc

cguguccaaa gggcacgaug ugaccaucua caagacgugg uaccucagcu cacgaggcga
 gguccagaug ugcaaagaac accggcccau acgcaacuuc acauugcagc accuucagca
 ccacggaagc caccugaaag ccaacgccag ccaugaccag cccagaagc augggcuaga
 gcuagcuucu gaccaccacg guaacuucuc uaucaccug cgcaauguga cccaagga
 cagcgccuc uacugcuguc uagugauaga auuaaaaaac caccaccag aacaacgguu
 cuacgggucc auggagcuac agguacaggc aggcaaaggc ucggggucca caugcauggc
 gucuauaugag caggacagug acagcaucac ggcu

COMP pentamerization domain mRNA sequence (human) (SEQ ID NO: 7)

gaccugggcc cgcagaugcu ucgggaacug caggaaacca acgcggcgcu gcaggacgug
 cgggagcugc ugccggcagca ggucaggag aucacguucc ugaaaaacac ggugauggag
 ugugacgcu gcggg

COMP pentamerization domain mRNA sequence (mouse) (SEQ ID NO: 8)

gaccu gggccacag
 augcugcag aacuucagga gacuaaugcg gcgcugcaag acgugagaga gcuguugcga
 cagcagguca aggagaucac cuuccugaag aauacgguga uggauguga ugcugcga

VISTA extracellular domain amino acid sequence (human) (SEQ ID NO: 9)

fkvatpys lyvpegqnv tltrllgpv
 dkghdvtfyk twyrssrgev qtcserpir nltfqlhlh hgghqaants hdlaqrhgle
 sasdhgnfs itmrnlild sglycclvve irhhsehrv hgamelqvqt gkdapsncvv
 ypsssqdsen ita

VISTA extracellular domain amino acid sequence (mouse) (SEQ ID NO: 10)

fkvttpys lyvpegqna tltrilgpv
 skghdvtiyk twylssrgev qmckehrpri nftlqlhlh gshlkanash dqpqkhglel
 asdhgnfsi tlrvtpdrds glycclviel knhhpeqrfy gsmelqvqag kgsstcmas
 neqdsdsita

COMP pentamerization domain amino acid sequence (human) (SEQ ID NO: 11)

dl gpqmlrelqe tnaalqdvre llrqvreit flkntvmecd acg

COMP pentamerization domain amino acid sequence (mouse) (SEQ ID NO: 12)

dla pqmlrelqet naalqdvrel llrqvkeif lkntvmecda cg

Codon Optimized mVISTA.COMP DNA sequence (mouse) (SEQ ID NO: 13)

AAGCTTGCCACCATGGAACCGACACCCTGCTGCTGTGGGTGCTGCTGCTGTGGGTG
 CCAGGCAGCACCGGC TTCAAAGTGACCACCCCTACAGCCTGTACGTGTGCCCC
 GAGGGCCAGAATGCCACCCTGACCTGTAGAATCCTGGGCCCGTGTCCAAGGGCCA
 CGACGTGACCATCTACAAGACCTGGTATCTGAGCAGCAGAGGCGAGGTGCAGATGT
 GCAAAGAGCACCGGCCCATCCGGAACCTTCACCCTGCAGCATCTGCAGCACCACGGC
 AGCCACCTGAAGGCCAATGCCAGCCACGACCAGCCTCAGAAGCACGGCCTGGAAC
 GGCTCTGACCACCACGGAACTTCAGCATCACCTGCGGAACGTGACCCCCAGAG
 ACAGCGGCCTGTACTGCTGTCTCGTGATCGAGCTGAAGAACCACCCCGAGCAGC
 GGTCTACGGCAGCATGGAACCTGCAGGTCCAGGCCGCAAGGGCAGCGGCTCTACT
 TGCATGGCCAGCAACGAGCAGGACAGCGACTCCATCACAGCC
 gatctggccccccagatgctgagagagctgcaggaaacaaacgccgccct

gcaggacgtgcgcgagctgctgagacagcaagtgaagagatcaccttcctgaagaacaccgtgatggaatgcga
cgcttgcggc GAGAACCTGTATTTTCAAGGC
 CACCACTGATGA
 CACCACTGATGA

non-bold = nucleotides that are not translated
 wavy underline = DNA sequencing encoding IgKappa secretion signal
 uppercase, no underline (black) = VISTA extracellular domain
 solid underlined (gray) = Spacer sequences
 lower case italicized = COMP pentamerization domain
 stippled underline = DNA sequence encoding a his-tag
 double underlined = stop codon
 bold = nucleotides that are translated

mVISTA.COMP amino acid sequence (mouse) (SEQ ID NO: 14)

METDTLLLVWLLLWVPGSTGEFKVTPPYSLYVCPEGQNATLTCRILGPVSKGHDVT
IYKTWYLSSRGEVQMCKEHRPIRNF TLQHLQHGHSHLKANASHDQPQKHGLELASDHHG
N
FSITLRNVTPRDSGLYCLVIELKNHHPEQRFYGSMEQLVQAGKSGSTCMASNEQSDS
ITA *dlapqmlrelqetnaalqdvrellrqvkeitflkntvmecdacg*
 HHHHHHHHH

wavy underline = IgKappa secretion signal
 Bold (black) = VISTA extracellular domain containing IgV domain
 lower case italicized = COMP pentamerization domain
 stippled underline = Histidine tag
 underlined (gray) = Spacer sequences

Full length VISTA amino acid sequence (human) (SEQ ID NO: 15)

ACCESSION Q9H7M9

1 mgvptaleag swrwgslfa iflaaslgpv **aafkvatpys lvcpeganv tltcrllapv**
 61 **dkahdvtyfk twvrssraev qtcserripir nltfadihlh haahgaants hdlagrhole**
 121 **sasdhgnfs itmrltld salycclvve irhhhsehry haamelavqt akdapsncvv**
 181 **vpsssdgsen ita** aalatga civgilclpl illvykqrq aasnrraqel vrmdsniggi
 241 enpgfeaspp aqgipeakvr hplsyvaqrq psesgrhlls epstplspg pgdvffpsld
 301 pvpdspnfev i

Bold = VISTA extracellular domain containing IgV domain (amino acids 31-193)

Full length VISTA amino acid sequence (mouse) (SEQ ID NO: 16)

ACCESSION Q9D659

1 mgvpavpeas sprwgtllla iflaasrglv **aafkvttpys lvcpegana tltcrilapv**
 61 **skahdvtyfk twylssraev qmckehrpri nftlqhlqhh qshlkanash dqpqkhalel**
 121 **asdhgnfsi tlrnvtprds glycclviel knhhpearfy qsmelqvqag kasqstcmas**
 181 **neqdsdsita** aalatgaciv gilclplll lvykqrqvas hrraqelvrmdsntqgienp
 241 gfettppfqg mpeaktrppl syvaqrqps sgryllsdps tplsppgpgd vffpsldpvp
 301 dspnseai

Bold = VISTA extracellular domain containing IgV domain (amino acids 31-193)

Full length COMP amino acid sequence (human) (SEQ ID NO: 17)

ACCESSION NP_000086

```

1      mvpdtacvll llaalgasg qgqspglgsdl gpgmlrelge tnaalqdvre llrqqvreit
61     flkntvmecda acgmqqsrvt glpsvrpllh capgfcpgv aciqtesgar cgpcpagftg
121    ngshctdvne cnaahpcfprv rcintspgfr ceacppgysg pthqgvglaf akankqvctd
181    inecetgqhn cvpnsvcint rgsfqcgpcq pgfvgdqasg cqrarqfcp dgspsecheh
241    adcvlerdgs rscvcavgwa gngilcgrdt dldgfpdekl rcperqcrkd ncvtvpnsgq
301    edvdrdgigd acdpdadgdg vpnekdnclp vnmpdqntd edkwgdacdn crsqknddqk
361    dtdqdggrda cdddidgdri rnqadncprv pnsdqkdsdg dgigdacdnc pqksnpdqad
421    vdhdvfgdac dsdqdqgdg hqdsrdncpt vpnsaqedsd hdgggdacdd dddndgvpds
481    rdncrlvpnp gqedadrdgv gdvcqddfa dkvvdkidvc penaevltld fraftvvld
541    pegdaqidpn wvlnqgrei vqtmnsdpgl avgytafngv dfegtfhvnt vtdddyagfi
601    fgyqdsssfy vmwkwmeqt ywqanpfrav aepgiqlkav ksstgpgeql malwhtgdt
661    esqvrllwkd prnvgwkdkk syrwflqhrp qvgvirvfy egpelvadsn vldttmrgrg
721    rlgvfcfsqe niiwanlyr cndtipedye thqlrqa

```

Bold = Pentamerization domain (amino acids 28-73)

Full length COMP amino acid sequence (mouse) (SEQ ID NO: 18)

ACCESSION NP_057894

```

1      mgptacvlvl alailratgq gqiplggdl a gpmirelget naalqdvrel llrqqvkeitf
61     lkntvmecda cgmmqpartpg lsvrvplca pgscfpgvvc setatgarcg pcppgytgng
121    shctdvnech ahpcfprvc intspgfhe acppgfsqpt hegvgltfak snkqvctdin
181    ecetgqhncv pnsvcvntg sfqcgpcqpg fvgdqtsgcq rrgqhfcpgd spspchekan
241    cvlerdgsrs cvcavgwagn gllcgrdtld dgfpdeklrc serqcrkdnc vtpnsgqed
301    vdrdgigdac dpdadgdgvp neqdnclprv npdqnsdsd kwgdacdnrc skknddqkdt
361    dldgrgdacd dddidgdrin vadncprvp fdqsdsgdg vgdacdnpcq kdnpdqrdvd
421    hdfvgdacs dqdqdgdhq dsrdncptvp nsaqdsdhd gkgdacddd dndgvpdsrd
481    nclrvnpgg edndrdgvgd acqgdfadk vidkidvcpe naevltldfr afqtvvldpe
541    gdaqidpnwv vlnqgmeivq tmnsdpglav gytanfngvdf egtfhvntat dddyagfig
601    yqdsssfyvv mwkwmeqtyw qanpfravae pgiqlkavks stgpgeqlrn alwhtgdtas
661    qvrllwkdpr nvgwkdksy rwlqhrpqv gyirvfyeg pelvadsnv ldtamrggrl
721    gvfcfsqeni iwanlyrcn dtipedyesh rlqrv

```

Bold = Pentamerization domain (amino acids 28-72)

VISTA cDNA nucleotide sequence (human) (SEQ ID NO: 19)

ACCESSION NM_022153

```

1      gggggcgggt gcctggagca cggcgctggg gccgcccga gcgctcactc gctcgactc
61     agtcgcggga ggcttccccg cgccggccgc gtcccgccg ctccccggca ccagaagtc
121    ctctgcgcgt ccgacggcga catgggcgtc cccacggccc tggaggccgg cagctggcgc
181    tggggatccc tgctcttcgc tctcttcctg gctgcgtccc taggtccggt ggcaggccttc

```

241 aaagtcacca caccatattc ccttatatgc tctcccgagg gacagaacct caccctcacc
 301 tcaagactct tgggcccctg gacaaaaagg caccatgtga ccttctacaa gacgtggtac
 361 cacaactcga ggggagaggt gacagacctc tcagagcgcc gggccatccg caacctcacc
 421 ttccaggacc ttacctgca ccatggaggc caccagggctg ccaacaccag ccacgacctg
 481 gctcagcgcc acgggctgga gtcggcctcc gaccaccatg gcaacttctc catcaccatg
 541 cgcaacctga cctgctgga tagcgccctc tactgctacc tgggtgtgga gatcaaggac
 601 caccactcga gacacagagt ccatggtgcc atggaagctc aagtcagac agcgaagagt
 661 gcaccatcca actatgtggt gtacccatcc tctcccagg atagtgaata catcacgact
 721 gcagccctgg ctacgggtgc ctgcatcgta ggaatcctt gctccccct catcctgctc
 781 ctggtctaca agcaaaggca ggcagcctcc aaccgcccgtg cccaggagct ggtgcggatg
 841 gacagcaaca ttcaagggtg tgaatacccc ggctttgaag cctcaccacc tgcccagggg
 901 ataccggagg ccaaagtcag gacccccctg tctatgtgg cccagcgggca gccttctgag
 961 tctggggcgc atctgcttc ggagcccagc acccccctgt ctctccagg ccccgagac
 1021 gtcttctcc catccctgga cctgtccct gactctcaa actttgaggt catctagccc
 1081 agctggggga cagtgggctg ttgtggctgg gtctggggca ggtgcattg agccagggct
 1141 ggctctgta gtggcctct tggcctcggc cctggttccc tccctcctgc tctgggtca
 1201 gatactgtga catccagaa gcccagcccc tcaaccctc tggatgtac atggggatgc
 1261 tggacggctc agcccctgtt ccaaggattt tgggggtgctg agattctccc ctgagacct
 1321 gaaattcacc agctacagat gccaatgac ttacatctta agaagtctca gaacgtccag
 1381 cccttcagca gctctcgtc tgagacatga gccttggat gtggcagcat cagtgggaca
 1441 agatggacac tggggccacc tcccaggcac cagacacagg gcacggtgga gagacttctc
 1501 ccccggtggc gccttggctc cccggtttg cccgaggctg ctcttctgc agacttctc
 1561 ttgtaccac agtggtctg gggccaggcc tgctgccc ctggccatcg ccacttccc
 1621 cagctgctc ctaccagcag ttctctgaa gatctgtca caggtaagt caatctggg
 1681 ctccactgc ctgattcca gtcccagag ctggtggct cggaaacggg aagtacat
 1741 tggggcatgg tggcctcgt gagcaaatgg tctctgggc aatctgaggc caggacagat
 1801 gttgcccac cactggaga tgggtctgag ggaggtgggt ggggccttct gggaaggta
 1861 gtggagagg gacctgccc cccgcccctc ccatcccta ctccactgc tcagcgcggtg
 1921 ccattgcaag ggtgccacac aatgtctgt ccaccctgg acacttctga gtatgaagc
 1981 ggatgtatt aaaaactaca tggggaaca ggtgcaaacc ctggagatgg attgtaagag
 2041 ccagttaaa tctgactct gctgctctc cccaccccc accctccact ccatacaatc
 2101 tgggctggt gtagtctcg ctccagagcc attcgccag gtgcgggtga tgtccatc
 2161 tctgctgtg ggcagccc tggcttgtt ttatacaca taggcaagg gtgctctg
 2221 tgaattgtg attgaaggat tttaagcag gggaggagag tagggggcat ctctgtacac
 2281 tctgggggta aaacaggga ggcagtgct gagcatggg acaggtgagg tgggctggg
 2341 cagaccccct gtacgttta gcaggatgg gggccagggt actgtggaga gcatagtcca
 2401 gctgggcat ttgtctcta gcagctaca ctggctctg ttagctggg ctgggtgctg
 2461 aaagccagga ttggggcta ggcgggaaga tttcgccca attgctggg gggttgggg
 2521 gatgaaaag gggagcacct ctaggctgc tggcagcagt gagccctgg cctgtggcta
 2581 cagccaggga acccacctg gacacatgg cctgttcta agccccag ttaggccaa
 2641 aggaatggt cactgaggg cctctgctt gcctgggctg ggccagggc ttgaggaga
 2701 gggtaaacat agggccggag atggggctga cactcgagt ggccagaata tgccaaacc
 2761 ccggttctc cttgtccct aggcagagg ggtccctt tttgtccc tctggtcacc
 2821 acaatgctg atgccagct ccataggaag aggggtgctg ctggccatgg tggcacacac
 2881 ctgtctccc agcacttgc agggctgagg tgaaggacc gcttaagccc aggtgtcaa
 2941 ggctgtgtg agctgtgtc gacccactac actccagct gggacggag caaaacttg
 3001 cctcaaaaaca aatttaaaa agaaagaaag aaggaaagag ggtatgttt tcacaattca
 3061 tgggggctg catggcagga tgggggacag gacacctgt gttctggag tcgaaggaca
 3121 agccacagc ccagattccg gttctccaa ctacaggaaga gcatgccctg cctctggg
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 3241 ttggctgcca gacccaggg ctggctgctg ctgtgtggag agggaggcgg cccgagcag
 3301 aacagccacc gacttctc ctacgttcc tctgtgctg cctgcccctc tcttctg
 3361 accctttac aactgaagc atctgggctt cgtgttcc tgtttcagc gaaatttact
 3421 ctgagctccc agttcatct tcatcatgg ccacaggccc tgcctacaac gcactaggga
 3481 cgtccctccc tgtgtgctg ggggagggg aggtgctg agccgccc ttagttgcc

3541 gggatggtag tgcctctgat gccagccctg gtggctgtgg gctgggggtgc atgggagagc
 3601 tgggtgcgag aacatggcgc ctccaggggg cgggaggagc actaggggct ggggcaggag
 3661 gctcctggag cgctggattc gtggcacagt ctgaggccct gagaggga aa tccatgcttt
 3721 taagaactaa ttcattgtta ggagatcaat caggaattag gggccatctt acctatctcc
 3781 tgacattcac agtttaatag agacttcctg cctttattcc ctcccaggga gaggtgaag
 3841 gaatggaatt gaaagcacca ttggagggt ttgctgaca cagcggggac tgctcagcac
 3901 tccctaaaaa cacaccatgg aggccactgg tgactgctgg tgggcaggct ggccttgctt
 3961 gggggagtcg gtggcgatgg gcgctgggt ggaggtgcag gagccccagg acctgctttt
 4021 caaaagactt ctgctgacc agagctccca ctacatgcag tggcccaggg cagaggggct
 4081 gatacatggc cttttcagg ggtgtcct cgcggggtgg acttgggagt gtgcagtggg
 4141 acagggggct gcaggggtcc tgccaccacc gagcaccaac ttggcccctg ggtcctgcc
 4201 tcatgaatga ggccttcccc agggctggcc tgactgtgct gggggctggg ttaacgtttt
 4261 ctcagggaac cacaatgcac gaaagaggaa ctgggggtgc taaccaggat gctgggaaca
 4321 aaggcctctt gaagcccagc cacagcccag ctgagcatga ggcccagccc atagacggca
 4381 caggccacct ggcccattcc ctgggcattc cctgctttgc attgtgctt ctctcacc
 4441 catggaggct atgtcacct aactatcctg gaatgtgtg agagggttc tgaatgatca
 4501 atatagcttg gtgagacagt gccgagatag atagccatgt ctgcttggg cacgggagag
 4561 ggaagtggca gcatgcacg tgtttcttg cttttctgt tagaatactt ggtgctttcc
 4621 aacacacttt cacatgtgt gtaactgtt tgatccacc cttccctga aaatcctggg
 4681 aggttttatt gctgccattt aacacagagg gcaatagagg ttctgaaagg tctgtgctt
 4741 gtcaaaacaa gtaaaccgtg gaactacgac taaa

Bold = VISTA extracellular domain containing IgV domain (235-720)

VISTA cDNA nucleotide sequence (mouse) (SEQ ID NO: 20)

ACCESSION XR_380449

1 catgaaggag ggcggagctg ggaggtgca aggtgctgt ggatctaggg aagaggaacc
 61 acagaaaagc taaaggagtc acctctgtct gtagcagcc **ttcaaggtca ccactccata**
 121 **ttctcttat gtgtgtcccg aqggacagaa tgccaccctc acctgcagga ttctgggccc**
 181 **ctgttccaaa gggcacgatg tgaccatcta caagacgtgg tacctcagct cacgagggca**
 241 **gttccagatg tgcaaaagac accggcccat acgcaacttc acattgcagc accttcaqca**
 301 **ccacggaaag cacctgaaag ccaacggcag ccatgaccag ccccaagaaac atggtctaga**
 361 **actaacttct gaccaccacg gtaacttctc tataccctg ccaatgtga ccccaagaaa**
 421 **cagcggcctc tactgtcttc tagtgataga attaaaaaac caccaccag aacaacggtt**
 481 **ctacgggtcc atggaactac aggtacagac aagcaaaagc tgggggtcca catgcatggc**
 541 **gtctaataag caggacagt acagcatcac ggcgtcgccc ctggccaccg gcgctgcat**
 601 cgtgggaatc ctctgcctcc ccttatcct gctgctgtc tataagcaga gacaggtggc
 661 ctctaccgc cgtgccagg agttggtgag gatggacagc aacaccaag gaatcgaaaa
 721 cccaggcttc gagaccactc caccctcca ggggatgctt gaggccaaga ccaggccgcc
 781 actgtctat gtggccagc ggcaacctc ggagtcagga cggtaacctg tcttgacc
 841 cagcacacct ctgtgcctc caggccctgg ggacgtctt tcccatccc tagatccagt
 901 cctgactcc cctaactctg aagccatcta aaccagctgg ggaacctga accatggtac
 961 ctgggtcagg gatattgca ctgatctat ggctggcctt tggacagtct tttaggcact
 1021 gactccagct tcttgctcc tgctctgagc ctgactctg cttttacaag atgcacagac
 1081 cctcccctat ctcttcaga cgctacttg ggggcaggga gaagatgtg gattgtcat
 1141 tgctgttctc aagatcttg gatgctgagt tctccctaga gacttgactt cgacagccac
 1201 agatgtcaga tgacctgcat cctatgaacg tccggcttg caagagcctt tctcatgga
 1261 aaccagtagc cggaggggga tgagggaact tggagccaca acatggaagg gacctgggct
 1321 gacaattgtc acaggaaagg ccaccactg gacacctgcc aactgctgg ctgagaagtc
 1381 ttgcatat gcatatccca tgaggactgc aacttggag gtgaagggtga agctgccgtt
 1441 ctgctcaag gacagggacc cacatgtgga acttgggtg ctggctcaag ttgggccttc
 1501 cattgccagg ctctactg aatatactc aacgactacc ctcaaagcc accccactaa

1561 aagccatggc tcctgggac tgactcatgg tagcaagtct caggacaccc cacactccag
 1621 atttctcat caggactgtg tgattaagca tcttctatta ttctttttc cacgggactg
 1681 gctgcagatg aacgtgaccc agtgcaatg agggctctgc acatggtagc tgacaataac
 1741 cagatgcccc cagagacttc cctggttgt tatggatgcc tgggaagctg aactggcccc
 1801 ccccccaaa tgtatgtacc aaggctgtac ctgccatgat gttctccagc acagacacga
 1861 agtaggccgg ctgctgaag gtgggagggt tgcgttctc atcctggaag gaaagcacat
 1921 ggatcagcat gtcgggaagg tactcggcca actttgggta aaagtaggaa ccgaggggga
 1981 gtttgggaag ggtggccctg agggctctg gcctctatgt atcaccagag gctagggtat
 2041 tatgaaagcc aggagtcctt ctgccaggtc agttgaagga taggtgggtt ggtctttggg
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 2161 agagcagtca gctcgtctcc agctccagat ctcccaccta acagctgcac tccaagctta
 2221 ctccagaggc ccagctccctt agttcttctg ttctatcca gcgtccacct gatctctccc
 2281 agctgagtca agacacagtg aaaggttcta gaagctctgc tagaatgaag agagaggggt
 2341 ggagagatgg cttaatggtc aagagcactg gctgctggtc cagaggactt ggttcaatt
 2401 cccagcatct acaactctct gtaactcag ttcccaggga tctgatgcct tttctggcc
 2461 tctgccattt ggcactcagc tggtagagag acaatccatt catgtaagca aaacacccaa
 2521 tcacatatat tttttaatt aatttaaaaa aggtgaggaa gaggattcaa gagagggtc
 2581 agcagtaaag aacactgtct gctctccag aggttcccg ttgaatccca gcacccacat
 2641 ggcagatcac aactatctgt aatttcagtt ccaggggac tggtagccct gcacagacat
 2701 acaatgcagg gaaaacatca atgtacattt aaaaaaatct tttttaaaag ggcgaaggag
 2761 aaaggcacat ctatgtagc agatatgaaa gtgtacaggg gcaggtagtg gtcaggctgg
 2821 tcccagagcc tggactggct gtagctggg ttggacagcc agcttggtct accagcttcg
 2881 catcctctgg atcacctgg tctgcagct ggtacctgcc agttccttc ttggctcag
 2941 cctgcagcag ctaggatgg gagtggaag cctggctgag gggctagacc aggtagagat
 3001 gagccctgtg ttggggagaa cccgtgccag gtgcagccca cagctcccta gactctcaa
 3061 tcagagaagc aagcctccca tcttgggac tctcaccaga gccaactct gccatcgctc
 3121 accgggaatt cctcccagtt gccttcttca ctgaagacgc tctggctccc ctgactccag
 3181 ctcaactccg tgcctccctc ctacgtcaa cagagcattg gaggagctca ggagctggct
 3241 gagtgtccg ccttctccc catgttgcaa tattcaatgg gcccactac taggtctca
 3301 gagctcaggg acctggagac tctccaggg gacagagttt gcatttgctg aactctttg
 3361 ctacggttct ttctgcatga gcaagcagga aaaggatgag accatgctgc ttggcccgcc
 3421 tctcccaga ctcgggacat cccagcctg ctgcatggtg tgtcccaga attgatttg
 3481 tccatttctt cccaaatctg tcccctgct ctgtcctgcg tacaggctca ctccctccc
 3541 aaggagctgg gagctttggg agtctctga ccccaagggt cccacagggt caccaggac
 3601 ctacccagg ctgctggcat gagtcaagt gctttgggta tacactggga ggcttctgt
 3661 cagctgcacg aagcaacgca gagaatgta gctacggagc acgaggcaga aacttattag
 3721 cgggtgatatt tcccggatt ctggcgctgt actgtctatt agttctaatt cagtggctc
 3781 ctctataaaa gcttattgtc ccctaattgg aaatct

Bold = VISTA extracellular domain containing IgV domain (101-574)

Full length COMP cDNA nucleotide sequence (human) (SEQ ID NO: 21)

ACCESSION NM_000095

1 agaaagcgag cagccaccca gctccccgcc accgccatgg tccccgacac cgctgcgtt
 61 ctctgtctca cctggctgc cctcggcgcg tccggacagg gccagagccc gttgggtca
 121 **gacctgggcc cgcagatgct tccggaaactg caggaaccca acacggcact gcaggacata**
 181 **caggagctgc tccggcagca ggtcagggag atcacattcc tgaataaacac ggtgattgag**
 241 **tatgacgctt acggg**atgca gcagtcagta cgcaccggcc taccagcgt gcggccctg
 301 ctccactgcg cgcccggtt ctgctcccc ggcgtggcct gcatccagac ggagagcgcc
 361 gcgcgtgcg gccctgccc cgcgggcttc acgggcaacg gctgcactg caccgacgtc
 421 aacgagtgc aagcccaccc ctgctcccc cgagtccgt gtatcaaac cagccgggg

481 ttccgctgcg aggccttggcc gccgggggtac agcggcccca cccaccaggg cgtggggctg
 541 gctttcgcca aggccaaaca gcaggtttgc acggacatca acgagtgtga gaccgggcaa
 601 cataactgcg tccccaactc cgtgtgcatc aacacccggg gctcctcca gtgcggcccg
 661 tgccagcccg gcttcgtggg cgaccaggcg tccggctgcc agcggcgcg acagcgcttc
 721 tgccccgacg gctcgccag cgagtgccac gagcatgcag actgctcct agagcgcat
 781 ggctcgcggt cgtgcgtgtg tgcgttggc tgggcccga acgggacct ctgtgtgcg
 841 gacactgacc tagacggctt cccggacgag aagctgcgt gcccgagcg ccagtgcct
 901 aaggacaact gcgtgactgt gccaactca gggcaggagg atgtggaccg cgatggcatc
 961 ggagacgcct gcgatccga tgcgacggg gacgggtcc ccaatgaaaa ggacaactgc
 1021 ccgctgggtc ggaaccaga ccagcgcaac acggacgagg acaagtggg cgatgcgtgc
 1081 gacaactgcc ggtcccaga gaacgacgac caaaggaca cagaccagga cggccggggg
 1141 gatgcgtgcg acgacgacat cgacggcgac cgatccgca accaggccga caactgccct
 1201 agggtagcca actcagacca gaaggacagt gatggcgatg gtataggga tgctgtgac
 1261 aactgtcccc agaagagcaa cccgatcag gcgatgtgg accacgactt tggggagat
 1321 gcttgtgaca gcgatcaaga ccaggatgga gacggacatc aggactctcg ggacaactgt
 1381 cccacgggtc ctaacagtgc ccaggaggac tcagaccacg atggccaggg tgatgcctgc
 1441 gacgacgacg acgacaatga cggagtcctt gacagtcggg acaactgccg cctgtgtcct
 1501 aaccccgcc aggaggacgc ggacaggac gccgtggcg acgtgtgcca ggacgacttt
 1561 gatgcagaca aggtgtaga caagatcgac gtgtgtccg agaacgctga agtcacgctc
 1621 accgacttca gggcctcca gacagtcgtg ctggaccgg aggtgacgc gcagattgac
 1681 ccaactggg tgggtctca ccagggaagg gagatcgtgc agacaatga cagcgacca
 1741 ggcttggtg tgggttacac tgccttcaat gccgtggact tcgagggcac gttccatgtg
 1801 aacacgttca cggatgacga ctatgcggc tcatctttg gctaccagga cagctccagc
 1861 ttctacgtg tcatgtgaa gcagatggag caaacgtatt ggcaggcgaa cccctccgt
 1921 gctgtggcg agcctggcat ccaactcaag gctgtgaagt ctccacagg ccccggggaa
 1981 cagctgcgga acgctctgt gcatacagga gacacagagt cccagggtcg gctgctgtg
 2041 aaggaccgc gaaacgtgg ttgaaggac aagaatcct atcgttgggt cctgcagcac
 2101 cggcccaag tgggtacat cagggtgca ttctatgagg gccctgagct ggtggccgac
 2161 agcaacgtg tcttgacac aacctgcgg ggtggccgc tgggggtct ctgttctc
 2221 caggagaaca tcatctggc caacctgcgt taccgtgca atgacacat cccagaggac
 2281 tatgagacc atcagctcg gcaagcctag ggaccagggt gaggaccgc cgatgacag
 2341 ccacctcac cgcggctga tgggggtct gacccagcc ccaaggggt gccgtccta
 2401 gggggaagt agaagggtc agagaggaca aaataaagt tgtgtcagg gaaaaaaaaa
 2461 aaaaaaaaaa a

Bold = Pentamerization domain (121-255)

COMP cDNA nucleotide sequence (mouse) (SEQ ID NO: 22)

ACCESSION NM_016685

1 gacagcagct gcagctccgc cgccatgggc cccactgcct gcgttctagt gctcgccctg
 61 gctatcttgc gggcgacagg ccaggggccag atcccgttg gtgga**gacct gacccacag**
 121 **atcttcgag aacttcagga gactaatgc gccttacaag acgtgagaga cctattcga**
 181 **cagcaggtca aggagatcac ctcttgaag aatacagatg tgaatatga tcttcgga**
 241 atgcagcccg cagcactcc aggcctgagc gtgcggccag tgcgctctg cgcacccggc
 301 tctgtcttc cggcgtagt ctgtccgag acagctacgg gcgcgcgtg cggccctgc
 361 cctctggct acaccggcaa cggctgcac tgcaccgacg ttaatgagt caatgtcac
 421 ccctgttcc cgcgggtgcg gtgcatcaat accagccctg gcttctactg cgaagcctgt
 481 cccctgggt tcagcggacc caccacgag ggcgtgggac tgaccttcg taagtccaac
 541 aaacaagttt gcacggatat taatgagtgt gagaccggg agcacaattg cgttccaac
 601 tccgtgtgcg tcaacaccg gggctcttc cagtgcggc cctgccagcc cggtttcgtg
 661 ggcgaccaga cgtcaggctg ccagcggcgt ggcagcact tctgccccga tgggtcaccc
 721 agcccggtcc atgagaaagc aaactgcgtc ctggagcggg atggctcgag gtcttgcgtg

781 tgtgcagttg gctgggcccg caacgggctc ctgtgcccgc gcgacacgga cctggacggt
 841 tttcctgacg agaagcttcg ctgctcagag cgccagtgtc gcaaggacaa ctgcgtgacg
 901 gtgcccgaatt cggggcagga ggatgtggac cgggacggca tcggagatgc ttgtgacccg
 961 gatgcccagc gggatggagt ccctaacgag caagacaatt gcccgctggt tcgaaacca
 1021 gaccagcgtg actcggacag tgataagtgg ggagatgcct gcgacaactg ccggtccaag
 1081 aagaatgacg atcagaaaaga tacagacctg gatggccggg gcgatgcctg cgacgacgac
 1141 atagatggcg accgaatacg aaatgtagct gacaactgtc cccgggtgcc caactttgac
 1201 cagagtgcga gtgatggtga tgggttggg gatgcctgtg acaactgtcc ccagaaagat
 1261 aaccagacc agagggatgt ggaccacgac tttgtgggtg atgcctgtga tagtgaccaa
 1321 gaccagatg gggatggta ccaggactcc cgggacaact gcccacagt acccaacagt
 1381 gccagcagg actcagatca tgatggcaag ggcgatgcct gtgatgacga tgatgacaat
 1441 gacggagttc ctgatagccg ggacaactgc cgcttggtgc ctaaccttg ccaagaggac
 1501 aatgaccggg atggcgtggg tgacgcgtgt cagggtgact tcgatgctga caaggttata
 1561 gacaagatcg atgtgtgcc cgagaacgcc gaggtcacc tcaccgactt cagggccttc
 1621 cagacggtg tgttgacccc cgagggtgat gcgacatcg atcccaactg ggtggtgctc
 1681 aatcaggga tggagatcgt tcagaccatg aacagtgacc ctggcctggc tgtgggttac
 1741 acagccttca acggcgtgga ctgcagggc acattccatg taaacaccgc cactgatgat
 1801 gactatgctg gttcatctt cggctacca gacagctcca gtttctact agtcattggt
 1861 aaacagatg agcagacgta ctggcaggcc aatcccttc ggcctgtggc tgagccaggg
 1921 attcagctca aggtgtcaa gtcctctaca ggtcccggg aacagctccg aaacgcactg
 1981 tggcacacgg gggacacagc atcccagggt cggctgctgt ggaaggatcc tcgaaacgtg
 2041 ggctggaagg ataaaacatc ctaccgctgg ttctgcagc accggcctca agttggctac
 2101 atcagggtgc ggttctatga gggcctgag ctatagctg acagcaatgt ggtgtggac
 2161 acggccatgc gtgtggccc cctgggtgtc ttctgcttct cccaagagaa catcatctg
 2221 gctaacctgc gctaccgtg caatgataca atccctgag actacgagag tcaccggctg
 2281 cagagagtct agggaccagt ggggtccgc tgctgatgg actgtggtg cacaagctac
 2341 ggggtgtgt gtgggggggt ctggcatccc tctgaagggg tcttggcct ggggaggaga
 2401 ggcaataaaa gtacgtatg ggggaaaaa aaaaaaaaa aaaaaaa

Bold = Pentamerization domain (105-240)

Codon Optimized VISTA.COMP DNA sequence (human) (SEQ ID NO: 23)

ATGGAACCGACACACTGCTGCTGTGGGTGCTGCTTCTTTGGGTGCCCGGAAGCACC
GGC TTCAAAGTGGCCACACCTTACAGCCTGTACGTGTGCCCTGAGGGCCAGAAT
GTGACCCTGACCTGTAGACTGCTGGGCCCCGTGGATAAGGGCCACGACGTGACCTTT
TACAAGACCTGGTACAGATCCAGCAGAGGCGAGGTGCAGACCTGCTCTGAGAGAAG
GCCCATCCGGAACCTGACCTTCCAGGACCTGCATCTGCACCACGGTGGACATCAGGC
CGCCAATACCTCTCATGATCTGGCCCAGAGACACGGCCTGGAAAGCGCCTCTGATCA
CCACGGCAACTTCAGCATCACCATGCGGAATCTGACCCTGCTGGACAGCGGCCTGTA
CTGCTGTCTGGTGGTGGAAATCAGACACCACACAGCGAGCACAGAGTGCACGGCG
CTATGGAAGTGCAGGTCCAGACAGGCAAGGACGCCCTAGCAATTGCGTGGTGTACC
CTAGCAGCAGCCAGGACAGCGAGAATATCACCGCC

gatctgggccctcagatgctgagagagctgcaagagacaaacgccgctctgcaggatgtg
cgcgagctgctgagacagcaagtgcgcgagatcaccttctgaagaacaccgtgatggaatgcgacgcctgtggc

CACCATCACCATCATCACCACTG

A

wavy underline = DNA sequencing encoding IgKappa secretion signal
 underlined (gray) = Spacer sequences
 uppercase, no underline (black) = VISTA extracellular domain
 lower case italicized = COMP pentamerization domain
 double underlined = stop codon

stippled underline = DNA sequence encoding a his-tag

VISTA.COMP amino acid sequence (human) (SEQ ID NO: 24)

METDTLLLWVLLWVPGSTGEfkvatpyslyvcpegqnvtltcrllgpvdkghdvtfyktwyrssrgevqtc
errpirnltfqdlhlhghghqaantshdlaqrhglesasdhhgnfsitmrnltldsglyccllveirhhhhsehrvhgam
elqvqtgkdapsncvvypsssqdsenita *dlgpqmlrelqetnaalqdvrellrqqvreitflkntvm*
ecdacg HHHHHHHHH

wavy underline = IgKappa secretion signal

lower case bold (black) = VISTA extracellular domain containing IgV domain

lower case italicized = COMP pentamerization domain

stippled underline = Histidine tag

underlined (gray) = Spacer sequences

B7-H4 extracellular domain amino acid sequence (human) (SEQ ID NO: 25)

ISGRHSITVTTVASAGNIGEDGILSCTFEPDIKLSDIVIQWLKEGVLGLVHEFK
 EGKDELSEQDEMFRGRTAVFADQVIVGNASRLKNVQLTDAGTYKCYIITSKGKGNANLE
 YKTGAFSMPEVNVNDYNASSETLRCEAPRWFPQPTVWWASQVDQGANFSEVSNTSFELNS
 E
 NVTMKVSVLYNVTINNTYSCMIENDIAKATGDIKVTSEIKRRSHLQLLSKAS

B7-H4 extracellular domain cDNA sequence (human) (SEQ ID NO: 26)

atttcagggagacactccatcacagtcactactgtcgctcagctgggaacattggggaggatggaatcctgagctgcacttttga
 acctgacatcaaactttctgatatcgatgatacaatggctgaaggaagggttttaggcttggtccatgagttcaaagaaggcaaag
 atgagctgtcggagcaggatgaaatgttcagaggccggacagcagtggttctgatcaagtatagttggaatgcctcttgcgg
 ctgaaaaacgtgcaactcacagatgctggcacctacaaatgttatcatcacttctaaaggcaagggaatgtaaccttgagt
 ataaaactggagccttcagcatgccggaagtgaatgttgactataatgccagctcagagaccttgcggtgtgaggctccccgat
 gggtccccagcccacagtggtctgggcatcccaagttgaccaggagccaactctcggaagtctcaataaccagcttgagct
 gaactctgagaatgtgacctgaaggtgtgtctgtctacaatgttaccgatcaacaacacatactcctgtatgattgaaatgac
 attgcaaagcaacaggggatatacaagtgacagaatcggagatcaaaaggcggagtcacctacagctgctaaactcaaag
 gcttct

B7-H4 extracellular domain mRNA sequence (human) (SEQ ID NO: 27)

auuucagggagacacuccaucacagucacucugucgcccucagcugggaacauuggggaggauuggaauccugagc
 ugcacuuuugaaccugacaucaaacuuucugauaucgugauacaauaggcugaaggaagguguuuuaggcuugguc
 caugaguucuaaagaaggcaaaugagcugucggagcaggauaaauguucagaggccggacagcaguguuugc
 ugaucagugauaguuggcaaugccucuuugcggcugaaaaacgugcaacucacagaucuggcaccuacaaug
 uuauaucacacucuaaaggcaagggggaugcuaaccuugaguauaaaacuggagccuucagcaugccggaagu
 gaauguggacuauaaugccagcucagagaccuugcggugugaggcucccgauugguuccccagcccacagugguc
 ugggcauccaaguugaccaggaggccaacuucucggaagucuccaaauaccagcuugagcugaacucugagaau
 gugaccaugaagguugugucugucucuaaauuuacgaucaacaacacauaccucuguaugauugaaaugac
 auugccaaagcaacaggggauaucaaaugagacagaauccggagaucaaaaggcggagucaccuacagcugcuaaac
 ucaaaggcuucu

B7-H4 full length amino acid sequence (human) (SEQ ID NO: 28)

ACCESSION Q7Z7D3

MASLGQILFWSIISIIILAGAIALIIGFGISGRHSITVTTVASAGNIGEDGILSCTFEP

DIKLSDIVIQWLKEGVLGLVHEFKEGKDELSEQDEMFRGRTAVFADQVIVGNASRLKKNV
 QLTDAGTYKCYIITSKGKGNANLEYKTGAFSMPEVNVNDYNASSETLRCEAPRWFPQPTVV
 WASQVDQGANFSEVSNTSFELNSENVTMKVVSVLYNVTINNTYSCMIENDIAKATGDIKV
 TESEIKRRSHLQLLNSKASLCVSSFFAISWALLPLSPYLMLK

B7-H4 full length cDNA nucleotide sequence (human) (SEQ ID NO: 29)

ACCESSION AY280972

1 atggcttccc tggggcagat cctcttctgg agcataatta gcatcatcat tattctggct
 61 ggagcaattg cactcatcat tggctttggt atttcagga gacactccat cacagtcact
 121 actgtcgct cagctgggaa cattggggag gatggaatcc tgagctgcac tttgaacct
 181 gacatcaaac ttctgatat cgtgatacaa tggctgaagg aagggtttt aggcttggtc
 241 catgagttca aagaaggcaa agatgagctg tcggagcagg atgaaatgt cagaggccgg
 301 acagcagtgt ttgctgatca atgatatgtt ggcaatgcct cttgcggct gaaaaacgtg
 361 caactcacag atgctggcac ctacaaatgt tatatcatca ctctaaagg caagggaat
 421 gtaaccttg agtataaaac tggagccttc agcatgccgg aagtgaatgt ggactataat
 481 gccagctcag agaccttgcg gtgtgaggct ccccgatgt tccccagcc cacagtggc
 541 tgggcatccc aagttgacca gggagccaac ttctcggaag tctcaatac cagcttgag
 601 ctgaactctg agaatgtgac catgaagggt gtgtctgtgc tctacaatgt tacgatcaac
 661 aacacatact cctgtatgat tgaaaatgac attgccaag caacagggga tatcaaagt
 721 acagaatcgg agatcaaaag gcggagtcac ctacagctgc taaactcaa ggcttctctg
 781 tgtgtctctt ctttcttgc catcagctgg gcaactctgc ctctcagccc ttacctgatg
 841 ctaaaataa

B7-H4.COMP amino acid sequence (human B7-H4.COMP) (SEQ ID NO: 30)

METDLLLLWVLLLWVPGSTG IGRHSITVTTVASAGNIGEDGILSCTFEPDIKLSDIV
 IQWLKEGVLGLVHEFKEGKDELSEQDEMFRGRTAVFADQVIVGNASRLKKNVQLTDAGT
 Y
 KCYIITSKGKGNANLEYKTGAFSMPEVNVNDYNASSETLRCEAPRWFPQPTVWASQVDQ
 G
 ANFSEVSNTSFELNSENVTMKVVSVLYNVTINNTYSCMIENDIAKATGDIKVTSEIKRR
 SHLQLLNSKA *dlapqmirelqetnaalqdvrellrqqvkeitflkntvm*
ecdacg HHHHHHHH

wavy underline = IgKappa secretion signal

Bold (black) = B7-H4 extracellular domain containing

lower case italicized = COMP pentamerization domain

stippled underline = Histidine tag

underlined (gray) = Spacer sequences

B7-H4.COMP codon optimized DNA sequence (human B7-H4) (SEQ ID NO: 31)

Atggagacagatacactcctgctctgggtcctcctgctgtgggtgcctggaagcacgggattcggcatttcagggcggcacagc
 atcacagtcactacggtagcagtgccgggaatattgggaagacggaatccttcatgtacctcgaacccgacataaagtgt
 cagatatcgtgatcaatggcttaagaaggagcttgggactcgttcacgagtttaaggaaggcaaagatgaactcagtgac
 aagacgagatgttaggggacggacagcgggtttgctgacaggtcattgtcggcaatgccagcctgctgcttaaaatgttcag
 ctcaccgatgcggggacacataagtgttatattatcacgtccaagggaagggtaacgcgaacctcgagtacaaaacaggagc
 ttttctatgcctgaagtgaatgtagactataatgcttcaagcgaactcttcgatgcgaagcgccacgctggttccgcagccact
 gtatgtggcgctcccaagtcgatcaggagcaaaacttcacgaggtgtcaaacacgtcatttgagcttaactctgagaacgtaa
 ccatgaaagtggtaagcgtgtgtataatgtcacaatcaataatactattcctgtatgatcgaaaacgatatcgctaaagcaaccg
 gcgacatcaaagttacggaatccgagattaagcgccgctcacacttgcaactccttaattccaaggccgaattcgggtcggccc

cggctctagcgggactgacctggctccgcaaatgcttagggagcttcaggaaacaaatgccgccttgcaagacgtgcgagaac
 tgctgagacaacaggttaaggagatcacattcctgaagaatacagttatggaatgtatgcctgcgggggtcccctcagcccc
 agtctgaaaattgtattccaggggggtccacagcccaaggtggttcaggctcaggctcagggtggccggcaccatcatcatcat
 caccaccactaatga

B7-H4.COMP amino acid sequence (mouse) (SEQ ID NO: 32)

METDTLLLWVLLLWVPGSTG LIIGFGISGKHFITVTTFTSAGNIGEDGTLSCTFEPDIKLN
GIVIQWLKEGIKGLVHEFKEGKDDLSQQHEMFRGRTAVFADQVVVGNASRLKNVQLTDA
GTYTCYIRTSKGKGNANLEYKTGAFSMPEINVDYNASSESLRCEAPRWFPQPTVAWASQ
VDQGANFSEVSNTSFELNSENVTMKVVSVLYNVTINNTYSCMIENDIAKATGDIKVTDSEVK
RRSQLQLLNSG *dlapqmlrelqetnaalqdvrellrqqvkeitflkntvm*
ecdacg HHHHHHHH

wavy underline = IgKappa secretion signal
 Bold (black) = B7-H4 extracellular domain containing
 lower case italicized = COMP pentamerization domain
 stippled underline = Histidine tag
 underlined (gray) = Spacer sequences

Codon optimized B7-H4.COMP amino acid sequence (mouse) (SEQ ID NO: 33)

ATGGAAACGACACCGCTGCTGCTGGGTGCTGCTGCTGGGTGCCAGGCAGCACC
GGC CTGATCATCGGCTTTGGCATCAGCGGCAAGCACTTCATCACCGTGACC
ACCTTTACCAGCGCCGGCAACATCGGCGAGGATGGCACACTGAGCTGCACCTTCGA
GCCCGACATCAAGCTGAACGGCATCGTGATCCAGTGGCTGAAAGAGGGCATCAAAG
GCCTGGTGCACGAGTTCAAAGAAGGCAAGGACGACCTGAGCCAGCAGCAGAGATG
TTCAGAGGCAGAACCGCCGTGTTCCGCCGATCAGGTGGTTCGTGGGAAATGCCAGCCT
GCGGCTGAAGAATGTGCAGCTGACAGACGCCGGCACCTACACCTGTTACATCCGGA
CCTCTAAAGGCAAGGGCAACGCCAACCTCGAGTACAAGACAGGCGCCTTCAGCATG
CCCGAGATCAACGTGGACTACAACGCCAGCAGCGAGAGCCTGAGATGCGAAGCCCC
TAGATGGTTCCTCAGCCTACAGTGGCTTGGGCTAGTCAGGTTGACCAGGGCGCCAA
CTTTAGCGAGGTGTCCAACACCAGCTTCGAGCTGAACAGCGAGAACGTGACCATGAA
GGTGGTGTCCGTGCTGTACAATGTGACCATCAACAACACCTACAGCTGCATGATCGA
GAACGATATCGCCAAGGCCACCGGCGACATCAAAGTGACCGACAGCGAAGTGAAGC
GGCGGAGCCAACTGCAGCTGCTGAATTCTGGA *gatctggccccccagatgctgagag*
agctgcaggaaacaaacgccgccctgcaggacgtgcgcgagctgctgagacagcaagtgaaagagatcaccttcc
tgaagaacaccgtgatggaatgcgacgcctgcggc

␣

ACCACCATCATCACCATCACCACCTGATGA

wavy underline = DNA sequencing encoding IgKappa secretion signal
 uppercase, no underline (black) = PD-L1 extracellular domain
 underlined (gray) = Spacer sequences
 lower case italicized = COMP pentamerization domain
 stippled underline = DNA sequence encoding a his-tag
 double underlined = stop codon

B7-H4 full length amino acid sequence (mouse) (SEQ ID NO: 34)

ACCESSION Q7TSP5

MASLGQIIFWSIINIILAGAIALIGFGISGKHFITVTTFTSAGNIGEDGTLSCTFEP
 DIKLNIGIVIQWLKEGIKGLVHEFKEGKDDLSQQHEMFRGRTAVFADQVVGNASRLKLV
 QLTDAGTYTCYIRTSKGKGNANLEYKTGAFSMPEINVDYNASSELRCEAPRWFPQPTVA
 WASQVDQGANFSEVSNTSFELNSENVTMKVSVLYNVTINNTYSCMIENDIAKATGDIKV
 TDSEVKRRSQLQLLNSGPSPCVFSSAFVAGWALLSLSCCLMLR

B7-H4 full length cDNA nucleotide sequence (mouse) (SEQ ID NO: 35)

ACCESSION AY280973

1 atggcttcct tggggcagat catcttttgg agtattatta acatcatcat catcctggct
 61 ggggccatcg cactcatcat tggctttggc atttcaggca agcacttcat cacggtcacg
 121 accttcacct cagctggaaa cattggagag gacgggaccc tgagctgcac ttttgaacct
 181 gacatcaaac tcaacggcat cgtcatccag tggctgaaag aaggcatcaa aggtttggct
 241 cacgagttca aagaaggcaa agacgacctc tcacagcagc atgagatgtt cagaggccgc
 301 acagcagtgt ttgctgatca ggtggtagt ggcaatgctt cctgagact gaaaaacgtg
 361 cagctcacgg atgctggcac ctacacatgt tacatccgca cctcaaaagg caaagggaat
 421 gcaaaccttg agtataagac cggagccttc agtatgccag agataaatgt ggactataat
 481 gccagttcag agagtttacg ctgcgaggct cctcgggtgt tccccagcc cacagtggcc
 541 tgggcatctc aagtcgacca aggagccaat ttctcagaag tctcaaacac cagctttgag
 601 ttgaactctg agaattgtac catgaaggtc gtatctgtgc tctacaatgt cacaatcaac
 661 aacacatact cctgtatgat tgaaaacgac attgccaaag ccaccgggga catcaaagt
 721 acagattcag aggtcaaaag gcgaagtcag ctgcagtgc tgaactctgg gcctccccg
 781 tgtgtttttt cttctgctt tgtggctggc tgggcactcc tatctctc ctgttgctg
 841 atgctaagat ga

PD-L1 extracellular domain amino acid sequence (human) (SEQ ID NO: 36)

FTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQH
 SSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKIN
 QRILVVDPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
 INTTNEIFYCTFRRLDPEENHTAELVPELPLAHPNER

PD-L1 extracellular domain cDNA nucleotide sequence (human) (SEQ ID NO: 37)

catttactgtcacgggtcccaaggacctatatgtggtagagtatggtagcaatatgacaattgaatgcaaattcccagtagaaaaa
 caattagacctggctgcactaattgtctattgggaaatggaggataagaacattattcaatttgcacatggagaggaagacctgaa
 gggtcagcatagtagctacagacagaggggcccggtgttgaggaccagctctccctgggaaatgctgcacttcagatcacaga
 tgtgaaattgcaggatgcaggggtgtaccgctgcatgatcagctatggtggtgccgactacaagcgaattactgtgaaagtcaat
 gccccatacaaaaaatcaaccaaagaattttggtgtggtaccagtcacctctgaacatgaactgacatgtcaggctgagggct
 accccaaggccgaagtcatctggacaagcagtgaccatcaagtcctgagtggtgaagaccaccaccaccattccaagagag
 aggagaagctttcaatgtgaccagcacactgagaatcaacacaactaatgagatttctactgcacttttaggagattagatc
 ctgaggaaaaccatacagctgaattggtcatccagaactacctctggcacatcctccaaatgaaagg

PD-L1 full length amino acid sequence (human) (SEQ ID NO: 38)

ACCESSION Q9NZQ7

MRIFAVFIFMTYWHLNFAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVYWEME
 DKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGG
 ADYKRITVKVNAPYNKINQRILVVDPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTT
 TNSKREEKLFNVTSTLRINTTNEIFYCTFRRLDPEENHTAELVPELPLAHPNER
 LVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

PD-L1 full length nucleotide sequence (human) (SEQ ID NO: 39)

ACCESSION AF177937

1 atgaggatat ttgctgtctt tatattcatg acctactggc atttctgaa cgcatttact
 61 gtcacgggtc ccaaggacct atatgtgga gagtatgga gcaatatgac aattgaatgc
 121 aaattccag tagaaaaaca attagacctg gctgactaa ttgtctattg ggaaatggag
 181 gataagaaca ttattcaatt tgtgcatgga gaggaagacc tgaagggtca gcatagtagc
 241 tacagacaga gggcccggct gttgaaggac cagctctccc tgggaaatgc tgcattcag
 301 atcacagatg tgaaattgca ggaatgcagg gtgtaccgct gcatgatcag ctatggtggt
 361 gccgactaca agcgaattac tgtgaaagtc aatgccccat acaacaaaat caaccaaaga
 421 attttggtg tggatccagt cacctctgaa catgaactga catgtcaggc tgagggtctac
 481 cccaaggccg aagtcattcg gacaagcagt gaccatcaag tcctgagtgg taagaccacc
 541 accaccaatt ccaagagaga ggagaagctt ttcaatgtga ccagcacact gagaatcaac
 601 acaacaacta atgagatttt ctactgcact tttaggagat tagatcctga ggaaaaccat
 661 acagctgaat tggatcatccc agaactacct ctggcacatc ctccaaatga aaggactcac
 721 ttgtaattc tgggagccat ctattatgc cttggtgtag cactgacatt catctccgt
 781 ttaagaaaag ggagaatgat ggaatgtgaaa aatgtggca tccaagatac aaactcaaag
 841 aagcaaagtg atacacattt ggaggagacg taa

PD-L1.COMP amino acid sequence (human) (SEQ ID NO: 40)

METDTLLLVVLLLWVPGSTG FTVTPKDLVVEYGSNM~~TIECKFPVEKQLDLAALIVYW~~
 EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMIS
 YGGADYKRITVKVNAPYNKINQRILVVDPTSEHELTCQAEGYPKAEVIWTS~~SDHQVLSGK~~
 TTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVPELPLAHPNER *dl*
apqmlrelqetnaalqdvrellrqvkeitflkntvmecdacg
 HHHHHHHHHH

wavy underline = IgKappa secretion signal

Bold (black) = PD-L1 extracellular domain containing IgV domain

lower case italicized = COMP pentamerization domain

stippled underline = Histidine tag

underlined (gray) = Spacer sequences

Codon optimized PD-L1.COMP encoding nucleotide sequence (human) (SEQ ID NO: 41)

ATGGAAACCGACACCCTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCAGGCAGCACC
 GGC **TTCACCGTGACCGTGCCTAAGGACCTGTACGTGGTGGGAATACGGCAGC**
AACATGACCATCGAGTGCAAGTTCCCCGTGGAAAAGCAGCTGGATCTGGCCGCTCTG
ATCGTGTACTGGGAGATGGAAGATAAGAACATCATCCAGTTCGTGCACGGCGAAGAG
GACCTGAAGGTGCAGCACAGCAGCTACAGACAGAGAGCCAGACTGCTGAAGGACCA
GCTGAGCCTGGGAAATGCCGCTCTGCAGATCACCGACGTGAAGCTGCAAGATGCCG
GCGTGTACCGGTGCATGATCTCTTATGGCGGAGCCGACTACAAGCGGATCACCGTGA
AAGTGAACGCCCCTTACAACAAGATCAACCAGCGGATCCTGGTGGTGGACCCTGTGA
CATCTGAGCACGAGCTGACCTGTCAGGCCGAGGGATATCCTAAGGCCGAAGTGATCT
GGACCAGCAGCGATCACCGGTGCTGAGCGGCAAGACCACCACCAACAGCAAG
CGGGAAGAGAAGCTGTTCAACGTGACCAGCACACTGCGGATCAACACAACCACCAA
CGAGATCTTCTACTGCACCTTTCGGCGGCTGGACCCCGAGGAAAATCACACAGCCGA
GCTGGTCATCCCCGAGCTGCCTCTTGCTCACCTCCTAACGAAAGA *gatctgg*
ccccccagatgctgagagagctgcaggaaacaaacgccgccctgcaggacgtgcgcgagctgctgagacagcaa
gtgaaagagatcaccttctgaagaacaccgtgatggaatgcgacgcctgcggc

CACCACCATCATCACCATCACCACCTGATGA

wavy underline = DNA sequencing encoding IgKappa secretion signal
 uppcase, no underline (black) = PD-L1 extracellular domain
 underlined (gray) = Spacer sequences
 lower case italicized = COMP pentamerization domain
 stippled underline = DNA sequence encoding a his-tag
 double underlined = stop codon

PD-L1 extracellular domain amino acid sequence (mouse) (SEQ ID NO: 42)

FTITAPKDLVVEYGSNVTMECRFPVERELDLLALVYWEKEDEQVIQFVAGEEDLKPQH
 SNFRGRASLPKDQLLKGNALQITDVKLQDAGVYCCIIISYGGADYKRITLKVNPYRKIN
 QRISVDPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRSVTTSRTEGMLLNVTSSLRV
 NATANDVFYCTFWRSQPGQNHTAELIPELPPATHPPQNR

PD-L1 extracellular domain cDNA nucleotide sequence (mouse) (SEQ ID NO: 43)

Ttactatcacggctccaaaggactgtacgtggtggagtatggcagcaacgtcacgatggagtgcagattccctgtagaacggg
 agctggacctgtctcggttagtgggtactgggaaaaggagaagatgagcaagtgattcagttgtggcaggagaggaggaccta
 agcctcagcacagcaacttcaggggagagcctcgctgccaaggaccagctttgaagggaatgctgccctcagatcaca
 gacgtcaagctgcaggcagcgagcggttactgctgcataatcagctacggtggtgcggactacaagcgaatcacgctgaaagt
 caatgccccataccgcaaatcaaccagagaattccgtggatccagccactctgagcatgaactaatatgcaggccgaggg
 ttatccagaagctgaggtaatctggacaaacagtgaccaccaaccggtgagtgggaagagaagtgaccactcccggaca
 gaggggatgcttctcaatgtgaccagcagctgagggtaacgccacagcgaatgatgtttctactgtacgtttggagatcacag
 ccaggggcaaaaccacacagcggagctgatcatccagaactgcctgcaacacatcctccacagaacagg

PD-L1 full length amino acid sequence (mouse) (SEQ ID NO: 44)

ACCESSION Q9EP73

MRIFAGIIFTACCHLLRAFTITAPKDLVVEYGSNVTMECRFPVERELDLLALVYWEKE
 DEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNALQITDVKLQDAGVYCCIIISYGG
 ADYKRITLKVNPYRKINQRISVDPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRSV
 TTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIPELPPATHPPQNRTHW
 VLLGSILLFLIVVSTVLLFLRKQVRMLDVEKCGVEDTSSKNRNDTQFEET

PD-L1 full length amino acid sequence (mouse) (SEQ ID NO: 45)

ACCESSION AF317088

1 atgaggatat ttgctggcat tatattcaca gcctgctgtc acttgctacg ggcgtttact
 61 atcacggctc caaaggactt gtacgtggtg gattatggca gcaacgtcac gatggagtgc
 121 agattccctg tagaacggga gctggacctg ctgctgtag tgggtactg ggaaaaggaa
 181 gatgagcaag tgattcagtt tgtggcagga gaggaggacc ttaagcctca gcacagcaac
 241 ttcaggggga gagcctcgct gccaaaggac cagctttga agggaaatgc tgccctcag
 301 atcacagacg tcaagctgca ggacgcaggc gtttactgct gcataatcag ctacggtggt
 361 gcggactaca agcgaatcac gctgaaagtc aatgccccat accgcaaaat caaccagaga
 421 atttccgtgg atccagccac ttctgagcat gaactaatat gtcaggccga gggttatcca
 481 gaagctgagg taatctggac aaacagtgac caccaaccgg tgagtgggaa gagaagtgtc
 541 accattccc ggacagaggg gatgcttctc aatgtgacca gcagtctgag ggtcaacgcc

601 acagcgaatg atgttttcta ctgtacgttt tggagatcac agccaggcca aaaccacaca
 661 gcggagctga tcatcccaga actgcctgca acacatcctc cacagaacag gactcactgg
 721 gtgcttctgg gatccatcct gttgttcctc attgtagtgt ccacggctct cctcttctg
 781 agaaaacaag tgagaatgct agatgtggag aaatgtggcg tgaagatac aagctcaaaa
 841 aaccgaaatg atacacaatt cgaggagacg taa

PD-L1.COMP amino acid sequence (mouse) (SEQ ID NO: 46)

METDLLLLWVLLWVPGSTG FTITAPKDLYVVEYGSNVTMECRFPVERELDLA
 LVVYWEKEDEQVIQFVAGEEDLKPDQHSNFRGRASLPKDQLLKGNALQITDVKLQDAGV
 Y
 CCIISYGGADYKRITLKVNPYRKINQRISVDPATSEHELICQAEGYPEAEVIWTNSDHQ
 PVSGKRSVTTSTRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIPELPATH
 PPQNR *dlapqmlrelqetnaalqdvrellrqvkeiflkntvmecdacg*
HHHHHHHHH

wavy underline = IgKappa secretion signal

Bold (black) = PD-L1 extracellular domain containing IgV domain

lower case italicized = COMP pentamerization domain

stippled underline = Histidine tag

underlined (gray) = Spacer sequences

PD-L1.COMP encoding nucleotide sequence (mouse) (SEQ ID NO: 47)

ATGGAAACCGACACCCTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCAGGCAGCACC
 GGC TTTACTATCACGGCTCCAAAGGACTTGTACGTGGTGGAGTATGGCAGC
 AACGTCACGATGGAGTGCAGATTCCCTGTAGAACGGGAGCTGGACCTGCTTGCGTTA
 GTGGTGTACTGGGAAAAGGAAGATGAGCAAGTGATTTCAGTTTGTGGCAGGAGAGGA
 GGACCTTAAGCCTCAGCACAGCAACTTCAGGGGGAGAGCCTCGCTGCCAAAGGACC
 AGCTTTTGAAGGGAAATGCTGCCCTTCAGATCACAGACGTCAAGCTGCAGGACGCAG
 GCGTTTACTGCTGCATAATCAGCTACGGTGGTGCGGACTACAAGCGAATCACGCTGA
 AAGTCAATGCCCCATACCGCAAAATCAACCAGAGAATTTCCGTGGATCCAGCCACTT
 CTGAGCATGAATAATATGTCAGGCCGAGGGTTATCCAGAAGCTGAGGTAATCTGGA
 CAAACAGTGACCACCAACCCGTGAGTGGGAAGAGAAGTGTCACCACTTCCCGGACA
 GAGGGGATGCTTCTCAATGTGACCAGCAGTCTGAGGGTCAACGCCACAGCGAATGAT
 GTTTTCTACTGTACGTTTTGGAGATCACAGCCAGGGGAAAACCACACAGCGGAGCTG
 ATCATCCCAGAACTGCCTGCAACACATCCTCCACAGAACAGG *gatctggcccc*
cagatgctgagagagctgcaggaaacaaacgccgccctgcaggacgtgcgcgagctgctgagacagcaagtga
agagatcaccttcctgaagaacaccgtgatggaatgcgacgcctgcggc

CACCACCATCATCACCATCACCCTGATGA

wavy underline = DNA sequencing encoding IgKappa secretion signal

uppercase, no underline (black) = PD-L1 extracellular domain

underlined (gray) = Spacer sequences

lower case italicized = COMP pentamerization domain

stippled underline = DNA sequence encoding a his-tag

double underlined = stop codon

ICOS-L extracellular domain cDNA nucleotide sequence (human) (SEQ ID NO: 48)

gatactcaggagaaggaagtcagagcgaatggttaggcagcgacgtggagctcagctgcgcttgcctgaaggaagccgttttga
 tttaaatgatgtttacgtatattggcaaacagtgagtcgaaaaccgtggtagctaccacatcccacagaacagctccttgaaa
 acgtggacagccgctaccggaaccgagccctgatgtcaccggccggcatgtctcggggagacttctccctgcgcttgttcaacg
 tcacccccaggagcagagcagaagtttactgcttggtgttgagccaatccctgggattccaggaggtttgagcgttgaggttaca
 ctgcatgtggcagcaaaacttcagcgtgcccgctcagcgccccccacagcccctccaggatgagctcaccttcagctgtacat
 ccataaacggctacccaggcccaacgtgtactggatcaataagacggacaacagcctgtggaccaggctctgcagaatga
 caccgtcttctgaacatgcggggctgtatgacgtggtcagcgtgctgaggatcgacggacccccagcgtgaacattggctgct
 gcatagagaacgtgcttctgcagcagaacctgactgtcggcagccagacaggaaatgacatcggagagagagacaagatca
 cagagaatccagtcagtaccggcgagaaaaacgcggccacgtggtcc

ICOS-L extracellular domain amino acid sequence (human) (SEQ ID NO: 49)

DTQEKEVRAMVGSDELSCACPEGSRFDLNDVYVYWQTSESKTVVYHIPQNSSLENVDS
 RYRNRALMSPAGMLRGDFSLRLFNVTPQDEQKFHCLVLSQSLGFQEVLSVEVTLHVAANF
 SVPVVSAPHSPSQDELFTCTTSINGYPRPNVYWINKTDNSLLDQALQNDTVFLNMRGLYD
 VVSVLRIARTPSVNIGCCIEENVLLQQNLTVGSQTGNDIGERDKITENPVSTGEKNAATWS

ICOS-L full length cDNA nucleotide sequence (human) (SEQ ID NO: 50)

ACCESSION AF289028

1 gagtagagcc gatctccgc gccccgaggt tgctctctc cgaggtctcc cgcgcccaa
 61 gttctccgcg ccccgaggtc tccgcgccc gaggtctcc cgccccgagg tctccgccc
 121 caccatgcgg ctgggcagtc ctggactgct ctctctgctc ttcagcagcc ttcgagctga
 181 tactcaggag aaggaaagtc gagcgtggt aggcagcgac gtggagctca gctgcgctg
 241 ccctgaagga agccgtttg atttaaatga tgtttacgta tattggcaaa ccagtgagtc
 301 gaaaaccgtg gtgacctacc acatcccaca gaacagctcc ttggaaaacg tggacagccg
 361 ctaccggaac cgagccctga tgcaccggc cggcatgctg cggggcgact tctccctgcg
 421 ctgttcaac gtcaccccc aggcagagca gaagttcac tgctgtgtg tgagccaatc
 481 cctgggattc caggaggttt tgagcgtga ggttactg catgtggcag caaacttcag
 541 cgtgcccgtc gtcagcgccc cccacagccc ctcccaggat gagctcacct tcacgtgtac
 601 atccataaac ggctacccca ggccaacgt gtactggatc aataagacgg acaacagcct
 661 gctggaccag gctctgcaga atgacaccgt ctcttgaac atgcggggct tgtatgacgt
 721 ggtcagcgtg ctgaggatcg cacggacccc cagcgtgaac attggctgct gcatagagaa
 781 cgtgctctg cagcagaacc tgactgtcg cagccagaca ggaaatgaca tcggagagag
 841 agacaagatc acagagaatc cagtcagtac cggcgagaaa aacgcggcca cgtggagcat
 901 cctggctgtc ctgtgcctgc ttgtggtcgt ggcggtggcc ataggctggg tgtcagggg
 961 ccgatgcctc caacacagct atgcaggtgc ctgggctgtg agtccggaga cagagctcac
 1021 tggccacgtt tgaccggagc tcaccgccc gagcgtggac agggcttcca tgagacgcca
 1081 ccgtgagagg ccagggtgga gcttgagcat ggactcccag actgcagggg agcacttgg
 1141 gcagccccc gaaggaccac tgctggatcc cagggagaac ctgctggcgt tggctgtgat
 1201 cctggaatga ggcccttca aaagcgtcat ccacacaaa ggcaaatgtc ccaagttag
 1261 tgggtcccc gctgtcactg ccagtcaccc acaggaaggg actggtgatg ggctgtctc
 1321 acccgagcg tgcgggatc agcaccaggc tctcccagt acccagacc cactgtggg
 1381 ctcccggtg gatcgggat cctgagaccg aagggtgtt ggtttaaaaa gaagactgg
 1441 cgtccgctc tccaggacgg cctctgtgt gctgggtca cgcaggctg ttgcagggg
 1501 acacggctac aggagctct ctgccctgaa cgctccaac ctgctccggc cggaagccac
 1561 aggaccact ca

ICOS-L full length amino acid sequence (human) (SEQ ID NO: 51)

ACCESSION O75144

MRLGSPGLLFLFSSLRADTQEKEVRAMVGSDELSCACPEGSRFDLNDVYVYWQTSESK

TVVTYHIPQNSSLENVDSRYRNRALMSPAGMLRGDFSLRLFNVTPQDEQKFHCLVLSQSL
 GFQEVLSVEVTLHVAANFSVPVVSAPHSPSQDELFTCTTSINGYPRPNVYWINKTDNSLL
 DQALQNDTVFLNMRGLYDVVSVLRIARTPSVNIGCCIEENVLLQQNLTVGSQTGNDIGERD
 KITENPVSTGEKNAATWSILAVLCLLVVAVAIGWVCRDRCLQHSYAGAWAVSPETELTG
 HV

ICOS-L extracellular domain cDNA nucleotide sequence (mouse) (SEQ ID NO: 52)

gagactgaagtcggtgcaatggtgggcagcaatgtggtgctcagctgcattgacccccacagacgccatttcaactgagtggtc
 tgtatgtctattggcaaatcgaaaaccagaagtttcggtgacttactacctgccttacaagctccagggaatcaatgtggacagttc
 ctacaagaacaggggccaatctgtccctggactccatgaagcagggttaacttctctgtacctgaagaatgcacccctcaggat
 acccaggagttcacatgccgggtatttatgaatacagccacagagttagtcagatcttgaagagggtggtcaggctgcgtgtgg
 cagcaaacttcagtacacctgtcatcagcacctctgatagctccaacccgggcccaggaaacgtacctacacctgcattgtccaaga
 atggctacccagagcccaacctgtattggatcaacacaacggacaatagcctaataagacacggctctgcagaataacactgtct
 acttgaacaagttggcctgtatgatgaatcagcacattaaggctcccttggacatctcgtgggatgttctgtctgcgtagagaa
 tgtggtctccaccagaacatcactagcattagccaggcagaaagtttactggaaataacacaaagaacccacaggaaacc
 cacaataatgagcttaag

ICOS-L extracellular domain amino acid sequence (mouse) (SEQ ID NO: 53)

ETEVGAMVGSNVVLSCIDPHRRHFNLSGLYVYWQIENPEVSVTYLPPYKSPGINVDSSYK
 NRGHLSLSMKQGNFSLYLKVNTPQDTQEFTCRVFMNTATELVKILEEVVRLRVAANFST
 PVISTSDSSNPGQERTYTCMSKNGYPEPNLYWINTTDSLIDTALQNNTVYLNKLGLYDV
 ISTLRPLWTSRGDVLCCVENVALHQNITSISQAESFTGNNTKNPQETHNNELK

ICOS-L full length amino acid sequence (mouse) (SEQ ID NO: 54)

ACCESSION Q9JHJ8

MLKPCPCFVSLGTRQPVWKKLHVSSGFFSGLGLFLLLLSSLCAASAETEVGAMVGSNVVLS
 CIDPHRRHFNLSGLYVYWQIENPEVSVTYLPPYKSPGINVDSSYKNRGHLSLSMKQGN
 FSLYLKVNTPQDTQEFTCRVFMNTATELVKILEEVVRLRVAANFSTPVISTSDSSNPGQE
 RYTCMSKNGYPEPNLYWINTTDSLIDTALQNNTVYLNKLGLYDVISTLRPLWTSRGDV
 LCCVENVALHQNITSISQAESFTGNNTKNPQETHNNELKVLVPVLAVLAAAAFVSFIYR
 RTRPHRSYTGPKTVQLELTDHA

ICOS-L full length cDNA nucleotide sequence (mouse) (SEQ ID NO: 55)

ACCESSION AF199027

1 ccggaacccc aaccgtgca actctccgcg tccgaaatcc agcatccgc agtctgcgt
 61 cgcaccatgc agctaaagt tccctgttt gtgtccttg gaaccaggca gcctgtttg
 121 aagaagctcc atgtttctag cgggttctt tctgtcttg gtctgttct gctgctgtg
 181 agcagcctct gtgtgcctc tgcagagact gaagtcggtg caatggtggg cagcaatgtg
 241 gtgtcagct gcattgacc ccacagacgc catttcaact tgagtgtgt gtatgtctat
 301 tggcaaatcg aaaaccaga agtttcggtg acttactacc tgccttaca gtctccagg
 361 atcaatgtgg acagttccta caagaacagg ggccatctgt ccctggactc catgaagcag
 421 ggtaaactct ctctgtacct gaagaatgtc acccctcagg ataccaggga gttcacatgc
 481 cgggtattta tgaatacagc cacagagta gtcaagatct tgaagaggt ggtcaggctg
 541 cgtgtggcag caaactcag tacacctgtc atcagcacct ctgatagctc caaccgggc
 601 caggaaacgta cctacacctg catgtccaag aatggctacc cagagcccaa cctgtattgg
 661 atcaacacaa cggacaatag cctaataagac acggctctgc agaataacac tgttacttg
 721 aacaagttgg gcctgtatga tgaatcagc acattaaggc tccctggac atctcgtgg
 781 gatgttctgt gctgcgtaga gaatgtggct ctccaccaga acatcactag cattagccag
 841 gcagaaagtt tactggaaa taacacaaag aaccacagg aaaccacaa taatgagta

Codon Optimized ICOS-L.COMP encoding nucleotide sequence (human) (SEQ ID NO: 56)

CGCCACCACCATCATCACCATCACCAC

TGATGA

wavy underline = DNA sequencing encoding IgKappa secretion signal
uppercase, no underline (black) = ICOS-L extracellular domain
underlined (gray) = Spacer sequences
lower case italicized = COMP pentamerization domain
stippled underline = DNA sequence encoding a his-tag
double underlined = stop codon

ICOS-L.COMP amino acid sequence (human) (SEQ ID NO: 57)

METDTLLLVWVLLWVPGSTG DTQEKEVRAMVGSDELSCACPEGSRFDLNDVYVYWQ
 T
 SESKTVVTYHIPQNSSLENVDSRYRNRALMSPAGMLRGDFSLRLFNVTPQDEQKFHCLVL
 SQSLGFQEVLSVEVTLHVAANFSPVVSAPHSPSQDELFTCTTSINGYPRPNVYWINKTD
 NSLLDQALQNDTVFLNMRGLYDVVSVLRIARTPSVNIGCCCIENVLLQQNLTVGSQTGNDI
 GERDKITENPVSTGEKNAATWS *dlapqmlrelqetnaalqdvrellrqg*
vkeitflkntvmecdacg HHHHHHHH

wavy underline = IgKappa secretion signal
 Bold (black) = ICOS-L extracellular domain containing IgV domain
 lower case italicized = COMP pentamerization domain
 stippled underline = Histidine tag
 underlined (gray) = Spacer sequences

Codon optimized ICOS-L.COMP nucleotide sequence (mouse) (SEQ ID NO: 58)

ATGGAAACCGACACCTTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCAGGCAGCACCGGC
GAACTGAAGTAGGAGCTATGGTCGGGTCTAACGTCGTCCTCTCCTGC
ATTGATCCGCACAGAAGGCATTTCAACTTGTACGGTCTGTACGTGTACTGGCAAATTG
AAAACCCGGAAGTTTCAGTTACATACTACTTGCCATATAAATCTCCTGGTATAAATGT
AGATAGCTCTTATAAAAATAGAGGACATCTCAGTCTGGATTCAATGAAACAAGGTAAC
TTCTCACTGTACCTTAAGAATGTAACGCCACAAGACACACAGGAATTTACGTGTAGGG
TATTTATGAACACTGCCACAGAACTGGTGAAAATACTTGAAGAGGTTGTGCGCCTGC
GCGTGGCGGCGAACTTTTCAACGCCTGTTATTTCAACTAGTGACAGTTCTAATCCTGG
ACAAGAACGAACGTATACCTGTATGTCCAAGAATGGTTACCCAGAGCCCAACCTTTAT
TGGATAAACACGACCGATAACAGCCTTATTGACACGGCGCTTCAGAACAAACACAGTG
TACCTTAACAAATTGGGATTGTATGACGTAATTTCCACGTTGAGACTTCCTTGGACTA
GTAGAGGAGACGTTTTGTGCTGCGTGGAGAATGTTGCTTTGCATCAAATATTACCTC
AATTTCTCAAGCGGAGTCTTTCACCGGCAATAATACCAAGAACCCACAAGAAACGCA
CAACAACGAGTTGAAA gatctggcccc
ccagatgctgagagagctgcaggaacaacgccgcctgcaggacgtgcgcgagctgctgagacagcaagtga
aagagatcaccttctgaagaacaccgtgatggaatgcgacgcctgcccc

CGCCACCACCATCATCACCATCACCACTGATGA

wavy underline = DNA sequencing encoding IgKappa secretion signal
 uppercase, no underline (black) = ICOS-L extracellular domain
 underlined (gray) = Spacer sequences
 lower case italicized = COMP pentamerization domain
 stippled underline = DNA sequence encoding a his-tag
 double underlined = stop codon

Codon optimized ICOS-L.COMP amino acid sequence (mouse) (SEQ ID NO: 59)

METDTLLLWVLLWVPGSTG **ETEVGAMVGSNVVLS****CIDPHRRHFNLSGLYVYWQIENP**
EVS**VTYYLPYKSPGINVDSSYKNRGHLSLDSMKQGNFSLYLKNVTPQDTQEFTCRVFMNT**
ATELVKILEEVRLRVAANFSTPVISTDSSNPGQERTYTCMSKNGYPEPNLYWINTTDN
SLIDTALQNNTVYLNKLGLYDVISTLRLPWTSRGDVLCCVENVALHQNITSISQAESFTG
NNTKNPQETHNNELK *dlapqmlrelqetnaalqdvrellrqqvkeitflkntvmecdacg*
HHHHHHHHH

wavy underline = IgKappa secretion signal

Bold (black) = ICOS-L extracellular domain containing IgV domain

lower case italicized = COMP pentamerization domain

stippled underline = Histidine tag

underlined (gray) = Spacer sequences

Strep-tag-II VISTA.COMP amino acid sequence (mouse) (SEQ ID NO: 60)

METDTLLLWVLLWVPGSTGEFKVTPYSLYVCPEGQNATLTCRILGPVSKGHDVTIYKTW
 YLSSRGEVQMCKEHRPIRNF~~TLQHLQH~~HGSHLKANASHDQPKHGLELASDHHGNFSITL
 RNVTPRDSGLYCCLVIELKNHHPEQRFYGSME~~LQVQAGK~~SGSGTCMASNEQDSDSITAEF
 GSGPGPSGTDLAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMECDACGGSPQPQ
 SENLYFQGGPQPQGGSGSGGLPETGGW~~SH~~PQFEK

Human ICOS-L extracellular domain mRNA sequence (SEQ ID NO: 61)

1 gaguagagcc gaucuccgcg gccccgaggu ugcuccucuc cgaggucucc cgcggcccaa
 61 guucuccgcg gccccgaggu uccgcgcccc gaggucuccg cggccccagg ucuccgcccc
 121 caccaugcgg cugggcaguc cuggacugcu cuuccugcuc uucagcagcc uucgagcuga
 181 uacucaggag aaggaaguca gagcgauggu aggcagcgac guggagcuca gcugcgcuug
 241 cccugaagga agccguuuug auuuaaauga uguuuacgua uauuggcaaa ccagugaguc
 301 gaaaaccgug gugaccuacc acauccaca gaacagcucc uuggaaaacg uggacagccg
 361 cuaccggaac cgagccuga ugucaccggc cggcaugcug cggggcgacu ucucccugcg
 421 cuuguucaac gucaccccc aggacgagca gaaguuuac ugccuggugu ugagccaau
 481 ccugggauuc caggagguuu ugagcguuga gguuacacug cauguggcag caaacuucag
 541 cgugcccguc gucagcgccc cccacagccc cucccaggau gagcucaccu ucacguguac
 601 auccauaaac ggcuaccca ggccaacgu guacuggauc aauaagacg acaacagccu
 661 gcuggaccag gcucugcaga augacaccgu cuucuugaac augcggggcu uguaugagcu
 721 ggucagcgug cugaggauug cacggacccc cagcgugaac auuggcugcu gcauagagaa
 781 cgugcuucug cagcagaacc ugacugucgg cagccagaca ggaaaugaca ucggagagag
 841 agacaagauc acagagaau cagucaguac cggcgagaaa aacgcggcca cguggagcau
 901 ccuggcuguc cugugccugc uuguggucgu ggcgguggcc auaggcuggg ugucagggga
 961 ccgaugccuc caacacagcu augcaggugc cugggcugug aguccggaga cagagcucac
 1021 uggccacguu ugaccggagc ucaccgccc gagcguggac agggcuucca ugagacgcca
 1081 ccugagagag ccagguggca gcuugagcau ggacucccag acugcagggg agcacuuggg
 1141 gcagccccc gaaggaccac ugcuggaucc cagggagaac cugcuggcgu uggcugugau
 1201 ccuggaauga ggcccuuuca aaagcgucac cacacacaaa ggcaaauguc ccaagugag
 1261 ugggcucccc gcugucacug ccagucaccc acaggaagg acuggugaug ggcugucucu
 1321 acccggagcg ugcgggauuc agcaccaggc ucuucccagu acccagacc cacugugggu
 1381 cuucccgugg gaugcgggau ccugagaccg aaggguuuu gguuuuuuu gaagacuggg
 1441 cguccgcucu ucaggacgg ccucugugcu gcuggguuca cgcgaggcug uuugcagggg
 1501 acacggucac aggagcucu cugcccuga cgcuuccaac cugcuccggc cggaagccac
 1561 aggaccacu ca

Human PD-L1 extracellular domain mRNA sequence (SEQ ID NO: 62)

uuuacuaucacggcuccaaaggacuuguacgugguggaguauggcagcaacgucacgauggagugcagauucccu
guagaacgggagcuggaccugcuugcguuagugguguacugggaaaaggaagagcaagugaauucaguuugu
ggcaggagaggaggaccuuagccucagcacagcaacuucagggggagagccucgcugccaaaggaccagcuuuu
gaagggaaaugcugcccuucagauacagacgucaagcugcaggacgcaggcguuuacugcugcauaaucagcua
cgguggugcggacuacaagcgaaucacgcugaaagucaaugcccauaccgcaaaaaucaaccagagaauuuccgu
ggauccagccacuucugagcaugaacuaauaugucaggccgaggguuauccagaagcugagguaaucuggacaaa
cagugaccaccaacccgugaguggaagagaagugucaccacuucccgacagaggggaugcuucucaaugugac
cagcagucugagggucaacgccacagcgaaugauguuuuuacuguaacguuuuggagauacagccagggcaaaa
ccacacagcggagcugaucauccagaacugccugcaacacauccuccacagaacagg

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CLAIMS

We claim:

1. A pentamerized polypeptide having T-cell stimulatory activity comprising:
 - five monomers, each of the monomers comprising:
 - a polypeptide having substantial similarity to the extracellular domain of ICOS-L (SEQ ID NO: 49) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).
2. The pentamerized polypeptide of claim 1, wherein the pentamerized polypeptide is in a soluble form.
3. The pentamerized polypeptide of claim 1, wherein the pentamerized polypeptide has increased T-cell stimulatory activity.
4. A recombinant polypeptide comprising:
 - a polypeptide having substantial similarity to the extracellular domain of ICOS-L (SEQ ID NO: 49) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).
5. The recombinant polypeptide of claim 4, wherein the recombinant polypeptide is in a soluble form.
6. A recombinant nucleic acid comprising:
 - a nucleic acid having substantial similarity to a nucleic acid encoding the extracellular domain of ICOS-L polypeptide, the nucleic acid having a sequence of SEQ ID NO: 48; and
 - a nucleic acid having substantial similarity to a nucleic acid encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 3, the nucleic acid encoding the extracellular domain of ICOS-L polypeptide being operably linked to the nucleic acid encoding the pentamerization domain of COMP.
7. An expression vector comprising the recombinant nucleic acid of claim 6.
8. The expression vector of claim 7, further comprising at least one control sequence.
9. A host cell comprising the expression vector of claim 7 or 8.

10. A pharmaceutical composition comprising one or more of:

- the polypeptide of any one of claims 4-5, the host cell of claim 9, and the pentamerized polypeptide of anyone of claims 1-3; and a pharmaceutically acceptable carrier, diluent, or excipient.

11. A method of eliciting a biological response in an individual in need thereof, the method comprising: administering to the individual a therapeutically effective amount of an ICOS-L-cartilage oligomeric matrix protein (COMP) fusion polypeptide (ICOS-L.COMP), the ICOS-L.COMP polypeptide having SEQ ID NO: 49 and being linked to SEQ ID NO: 11, wherein the biological response is one or more of:

- stimulation of T-cell activation;
- stimulation of T-cell proliferation;
- increased secretion by T-cells of one or more inflammatory cytokines;
- increased induction of cytotoxic T lymphocytes (CTLs); and

- an increase in the effector T-cells:regulatory T-cell ratio within the tumor microenvironment.

12. The method of claim 11, wherein the ICOS-L.COMP polypeptide is administered in combination with a checkpoint blocking molecule.

13. The method of claim 12, wherein the ICOS-L.COMP polypeptide is administered simultaneously with, or before, or after the checkpoint blocking molecule.

14. The method of claim 12, wherein the checkpoint blocking molecule is an anti-PD-1 antibody or an anti-CTLA-4 antibody.

15. A pentamerized polypeptide having T-cell inhibitory activity comprising:

- five monomers, each of the monomers comprising:
 - a polypeptide having substantial similarity to the extracellular domain of a V-domain Ig Suppressor of T cell Activation (VISTA) (SEQ ID NO: 9) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).

16. The pentamerized polypeptide of claim 15, wherein the pentamerized polypeptide is in a soluble form.

17. The pentamerized polypeptide of claim 16, wherein the soluble form pentamerized polypeptide has increased T-cell inhibitory activity relative to a soluble dimerized polypeptide comprising the extracellular domain of VISTA (SEQ ID NO: 9).

18. The pentamerized polypeptide of claim 17, wherein the increased T-cell inhibitory activity comprises one or more of increased inhibition of T-cell activation and T-cell proliferation.

19. The pentamerized polypeptide of claim 16, wherein the soluble form pentamerized polypeptide has increased immune inhibitory activity *in vivo* relative to a soluble dimerized polypeptide comprising the extracellular domain of VISTA (SEQ ID NO: 9).

20. The pentamerized polypeptide of claim 19, wherein the increased immune inhibitory activity comprises one or more of increased inhibition of cytokine secretion and cytotoxic lymphocyte (CTL) production.

21. A recombinant polypeptide comprising:

- a polypeptide having substantial similarity to the extracellular domain of a V-domain Ig Suppressor of T cell Activation (VISTA) (SEQ ID NO: 9) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).

22. The recombinant polypeptide of claim 21, wherein the recombinant polypeptide is in a soluble form.

23. A recombinant nucleic acid comprising:

- a nucleic acid having substantial similarity to a nucleic acid encoding the extracellular IgV containing domain of a V-domain Ig Suppressor of T cell Activation (VISTA) polypeptide, the nucleic acid having a sequence of SEQ ID NO: 1; and

- a nucleic acid having substantial similarity to a nucleic acid encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 3, the nucleic acid encoding the extracellular IgV containing domain of the VISTA polypeptide being operably linked to the nucleic acid encoding the pentamerization domain of COMP.

24. An expression vector comprising the recombinant nucleic acid of claim 23.
25. The expression vector of claim 24, further comprising at least one control sequence.
26. A host cell comprising the expression vector of claim 24 or 25.
27. A pharmaceutical composition comprising one or more of:
- the polypeptide of any one of claims 21-22, the host cell of claim 26, and the pentamerized polypeptide of anyone of claims 15-20; and a pharmaceutically acceptable carrier, diluent, or excipient.
28. A method of eliciting a biological response in an individual in need thereof, the method comprising: administering to the individual a therapeutically effective amount of a V-domain Ig Suppressor of T cell Activation (VISTA)-cartilage oligomeric matrix protein (COMP) fusion polypeptide (VISTA.COMP), the VISTA.COMP polypeptide having SEQ ID NO: 9 and being linked to SEQ ID NO: 11, wherein the biological response is one or more of:
- suppression of T-cell activation;
 - suppression of T-cell proliferation;
 - decreased secretion by T-cells of one or more inflammatory cytokines;
 - suppressed induction of cytotoxic T lymphocytes (CTLs); and
 - an increase in T-cells with regulatory phenotypes.
29. The method of claim 28, wherein the inflammatory cytokines comprise one or more of IL-2 and IFN γ .
30. A pentamerized polypeptide having T-cell inhibitory activity comprising:
- five monomers, each of the monomers comprising:
 - a polypeptide having substantial similarity to the extracellular domain of B7-H4 (SEQ ID NO: 25) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).
31. The pentamerized polypeptide of claim 30, wherein the pentamerized polypeptide is in a soluble form.

32. The pentamerized polypeptide of claim 30, wherein the pentamerized polypeptide has increased T-cell inhibitory activity.

33. A recombinant polypeptide comprising:

- a polypeptide having substantial similarity to the extracellular domain of B7-H4 (SEQ ID NO: 25) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).

34. The recombinant polypeptide of claim 33, wherein the recombinant polypeptide is in a soluble form.

35. A recombinant nucleic acid comprising:

- a nucleic acid having substantial similarity to a nucleic acid encoding the extracellular domain of B7-H4 polypeptide, the nucleic acid having a sequence of SEQ ID NO: 26; and

- a nucleic acid having substantial similarity to a nucleic acid encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 3, the nucleic acid encoding the extracellular domain of B7-H4 polypeptide being operably linked to the nucleic acid encoding the pentamerization domain of COMP.

36. An expression vector comprising the recombinant nucleic acid of claim 35.

37. The expression vector of claim 36, further comprising at least one control sequence.

38. A host cell comprising the expression vector of claim 36 or 37.

39. A pharmaceutical composition comprising one or more of:

- the polypeptide of any one of claims 33-34, the host cell of claim 38, and the pentamerized polypeptide of anyone of claims 30-32; and a pharmaceutically acceptable carrier, diluent, or excipient.

40. A method of eliciting a biological response in an individual in need thereof, the method comprising: administering to the individual a therapeutically effective amount of a B7-H4-cartilage oligomeric matrix protein (COMP) fusion polypeptide (B7-H4.COMP), the B7-H4.COMP polypeptide having SEQ ID NO: 25 and being linked to SEQ ID NO: 11, wherein the biological response is one or more of:

- suppression of T-cell activation;

- suppression of T-cell proliferation;
- decreased secretion by T-cells of one or more inflammatory cytokines;
- suppressed induction of cytotoxic T lymphocytes (CTLs); and
- an increase in T-cells with regulatory phenotypes.

41. A pentamerized polypeptide having T-cell inhibitory activity comprising:

- five monomers, each of the monomers comprising:

- a polypeptide having substantial similarity to the extracellular domain of PD-L1 (SEQ ID NO: 36) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).

42. The pentamerized polypeptide of claim 41, wherein the pentamerized polypeptide is in a soluble form.

43. The pentamerized polypeptide of claim 41, wherein the pentamerized polypeptide has increased T-cell inhibitory activity.

44. A recombinant polypeptide comprising:

- a polypeptide having substantial similarity to the extracellular domain of PD-L1 (SEQ ID NO: 36) linked to a polypeptide having substantial similarity to a pentamerization domain of cartilage oligomeric matrix protein (COMP) (SEQ ID NO: 11).

45. The recombinant polypeptide of claim 44, wherein the recombinant polypeptide is in a soluble form.

46. A recombinant nucleic acid comprising:

- a nucleic acid having substantial similarity to a nucleic acid encoding the extracellular domain of PD-L1 polypeptide, the nucleic acid having a sequence of SEQ ID NO: 37; and

- a nucleic acid having substantial similarity to a nucleic acid encoding a pentamerization domain of cartilage oligomeric matrix protein (COMP) having a sequence of SEQ ID NO: 3, the nucleic acid encoding the extracellular domain of PD-L1 polypeptide being operably linked to the nucleic acid encoding the pentamerization domain of COMP.

47. An expression vector comprising the recombinant nucleic acid of claim 46.

48. The expression vector of claim 47, further comprising at least one control sequence.
49. A host cell comprising the expression vector of claim 47 or 48.
50. A pharmaceutical composition comprising one or more of:
- the polypeptide of any one of claims 44-45, the host cell of claim 49, and the pentamerized polypeptide of anyone of claims 41-43; and a pharmaceutically acceptable carrier, diluent, or excipient.
51. A method of eliciting a biological response in an individual in need thereof, the method comprising: administering to the individual a therapeutically effective amount of a PD-L1-cartilage oligomeric matrix protein (COMP) fusion polypeptide (PD-L1.COMP), the PD-L1.COMP polypeptide having SEQ ID NO: 36 and being linked to SEQ ID NO: 11, wherein the biological response is one or more of:
- suppression of T-cell activation;
 - suppression of T-cell proliferation;
 - decreased secretion by T-cells of one or more inflammatory cytokines;
 - suppressed induction of cytotoxic T lymphocytes (CTLs); and
 - an increase in T-cells with regulatory phenotypes.

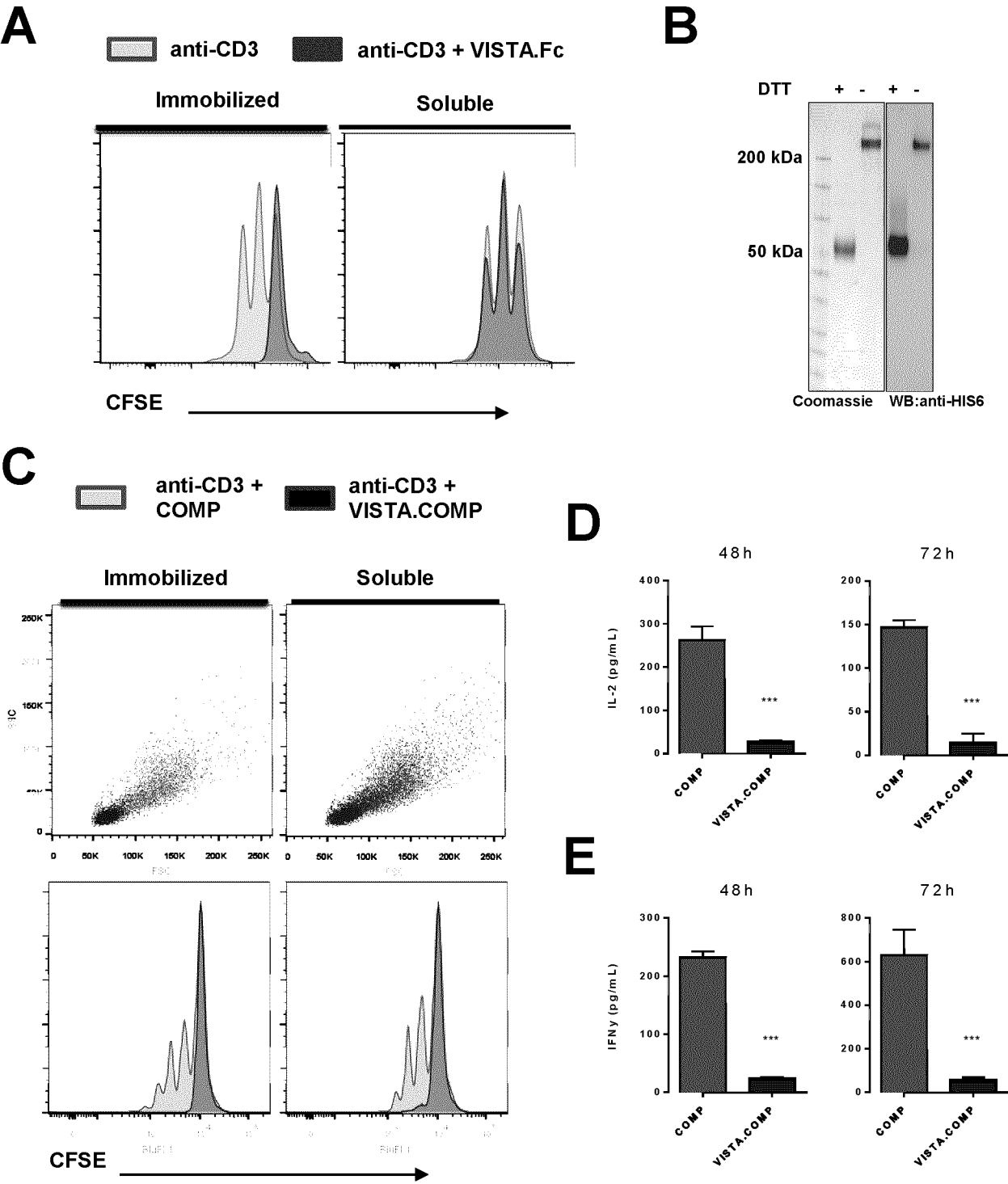
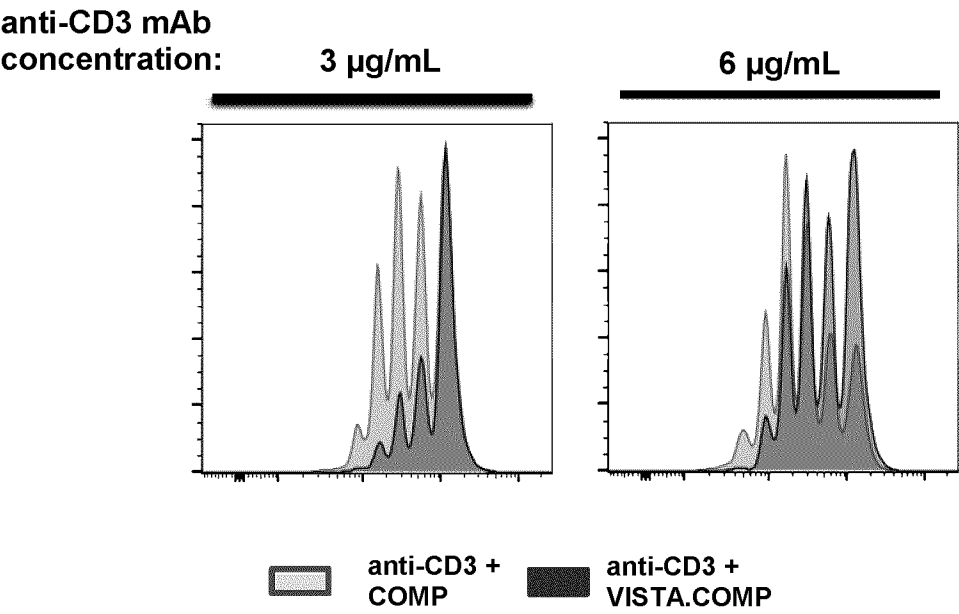


Figure 1 A-E

F



G

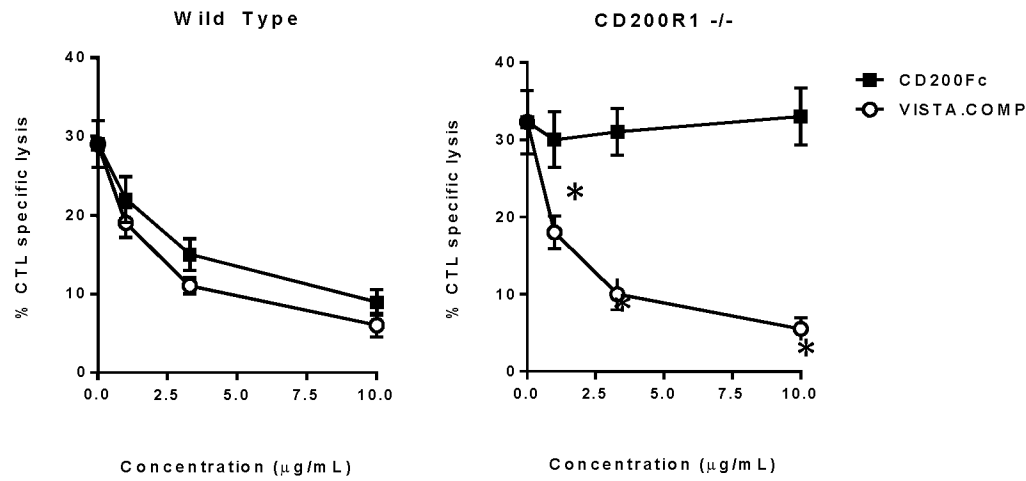


Figure 1 F-G

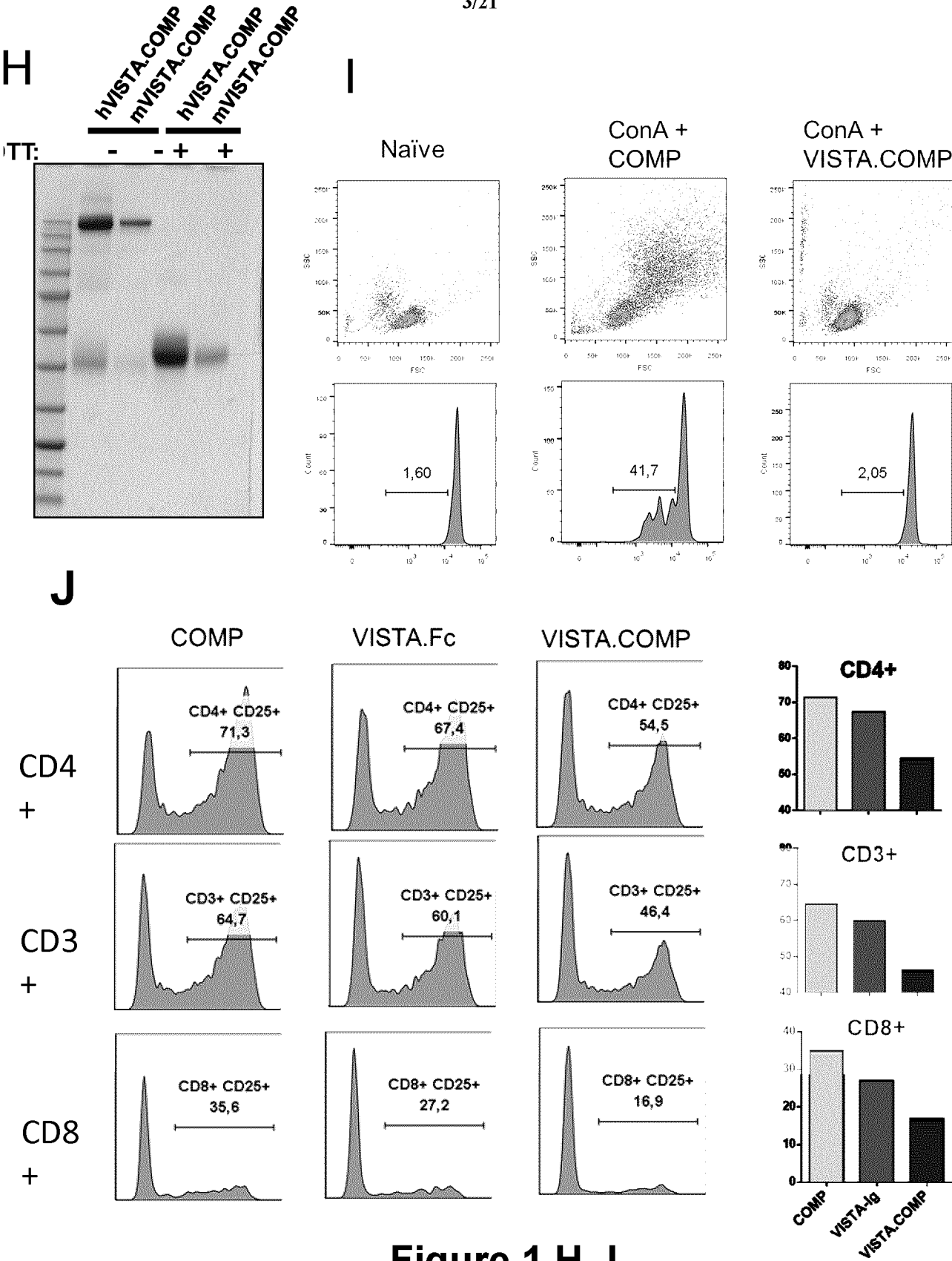


Figure 1 H-J

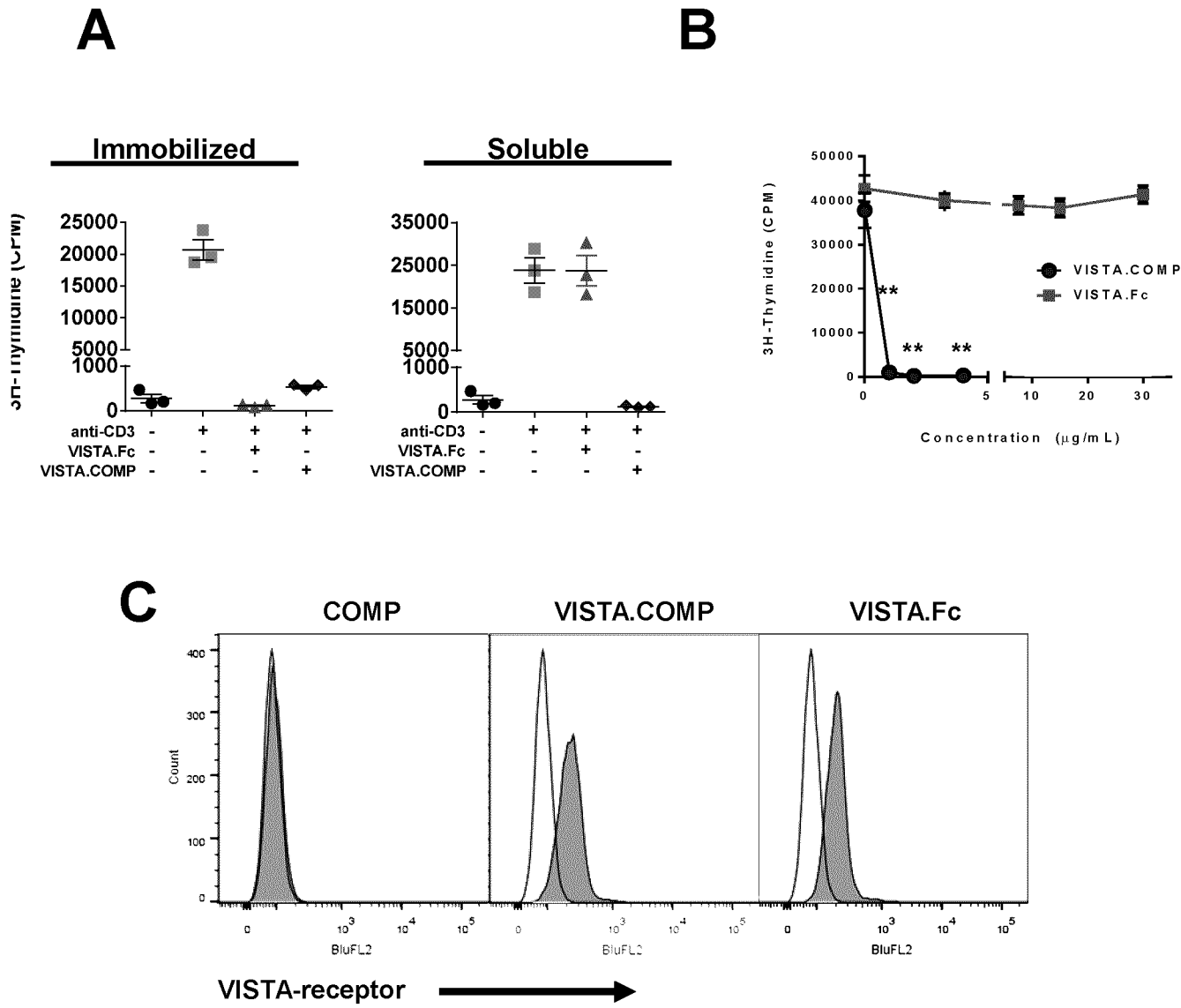


Figure 2 A-C

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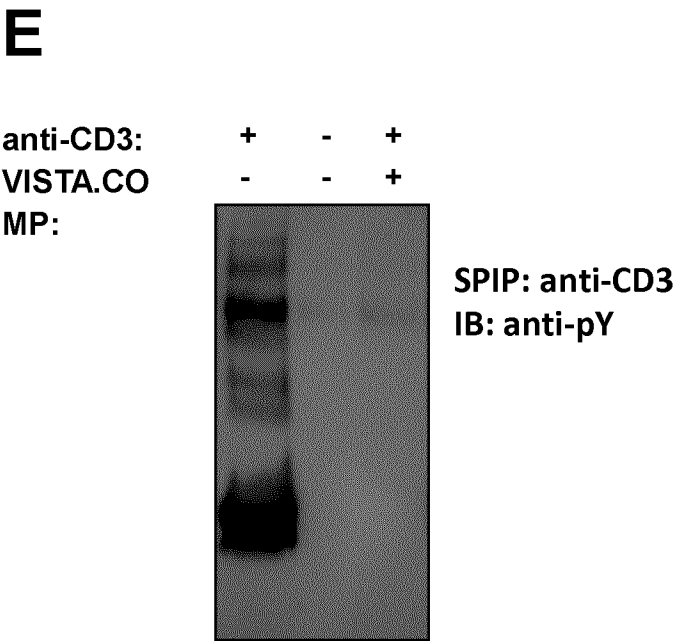
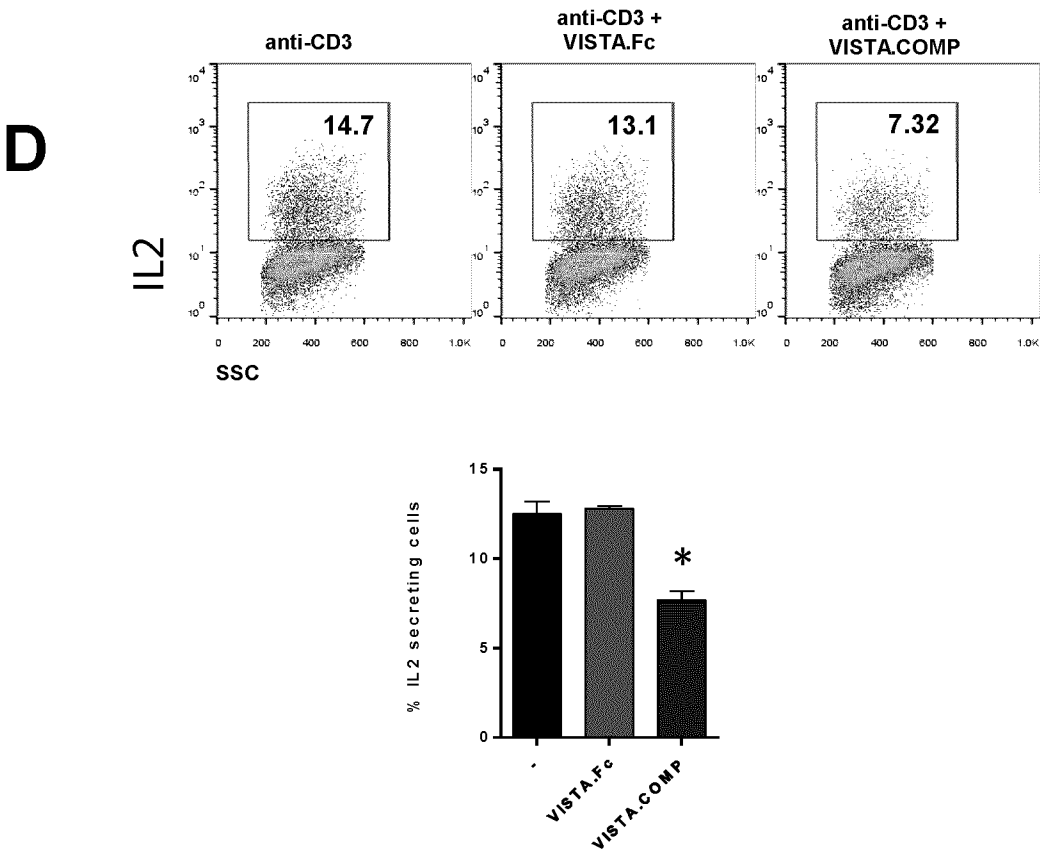


Figure 2 D-E

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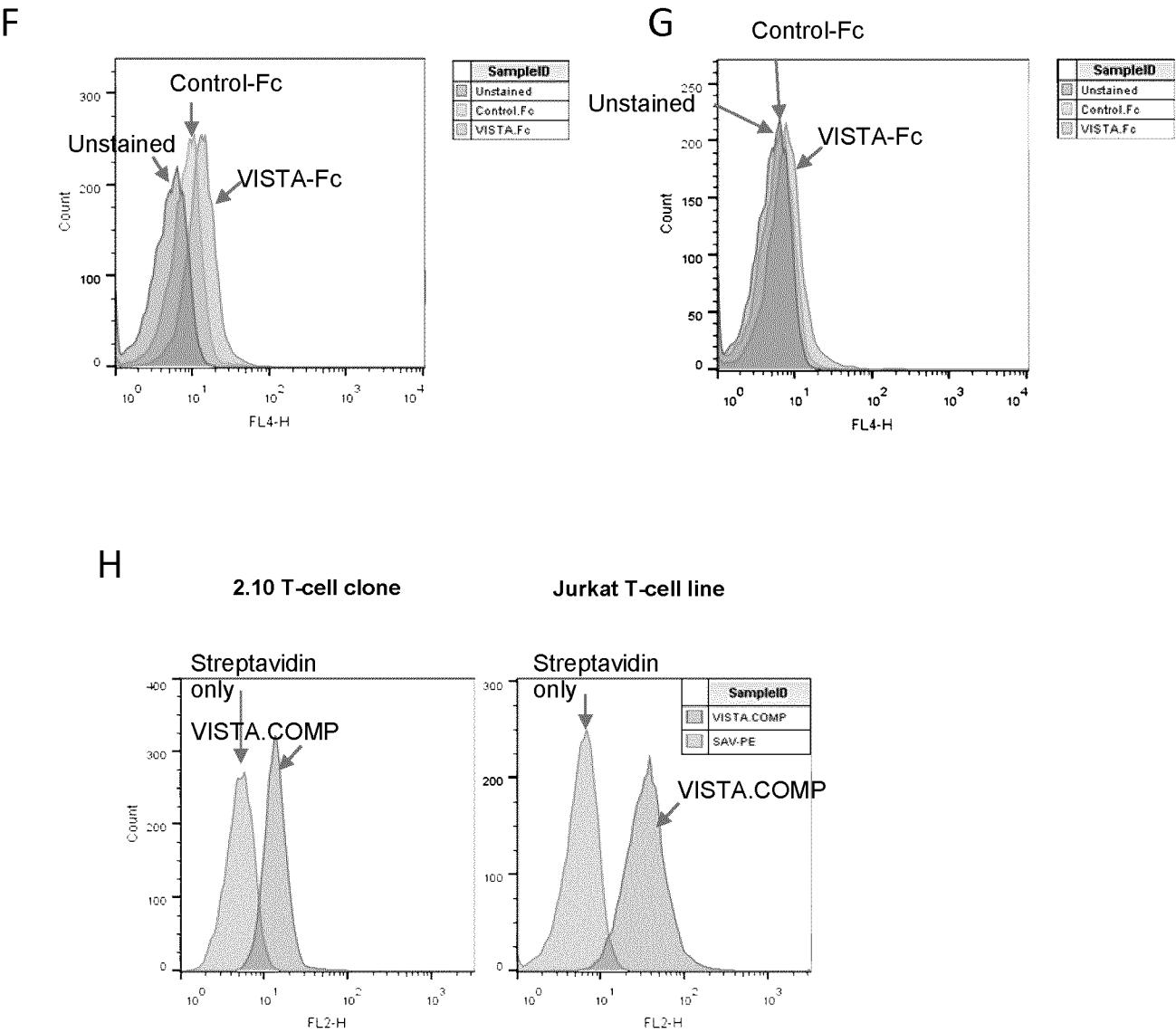


Figure 2 F-H

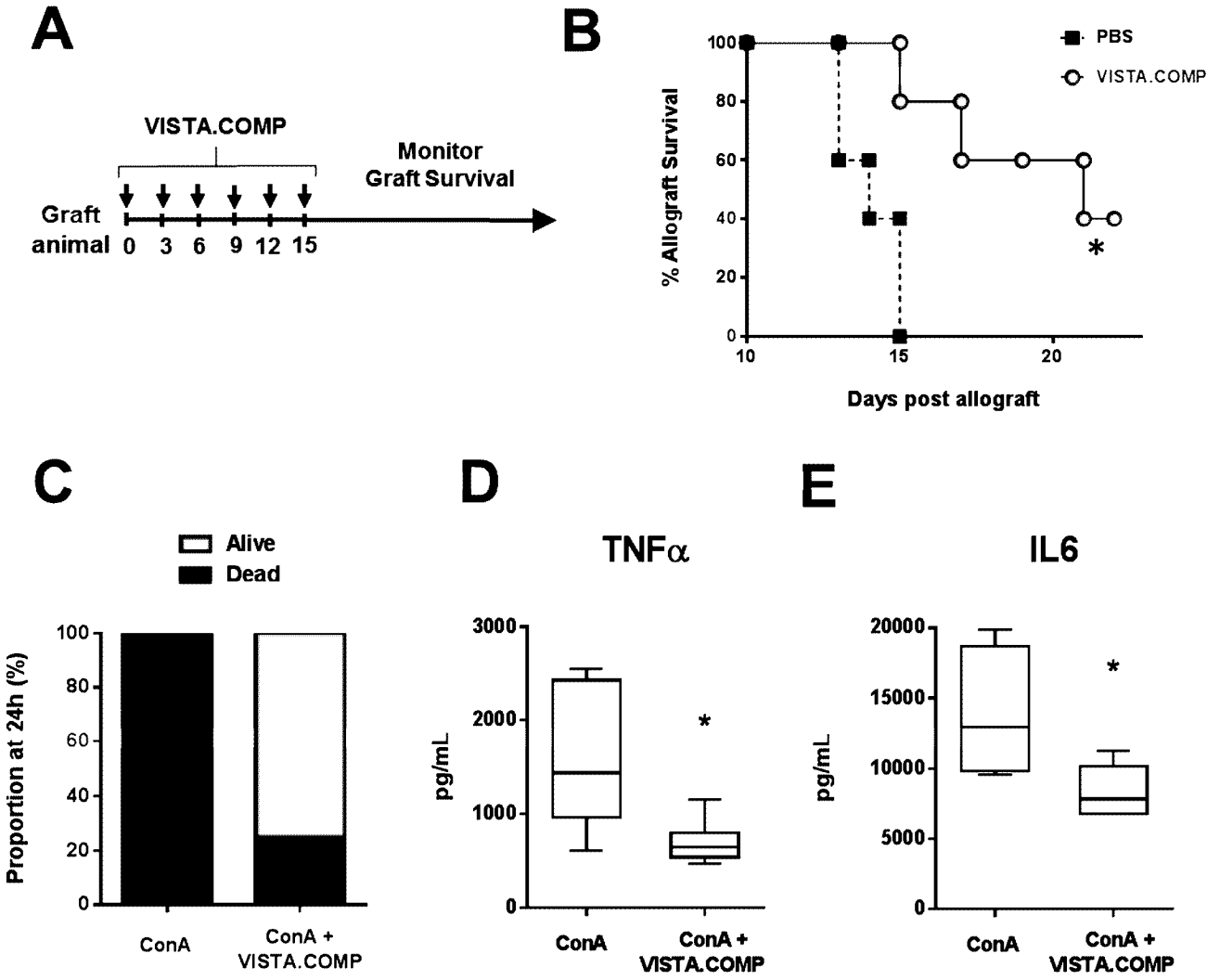
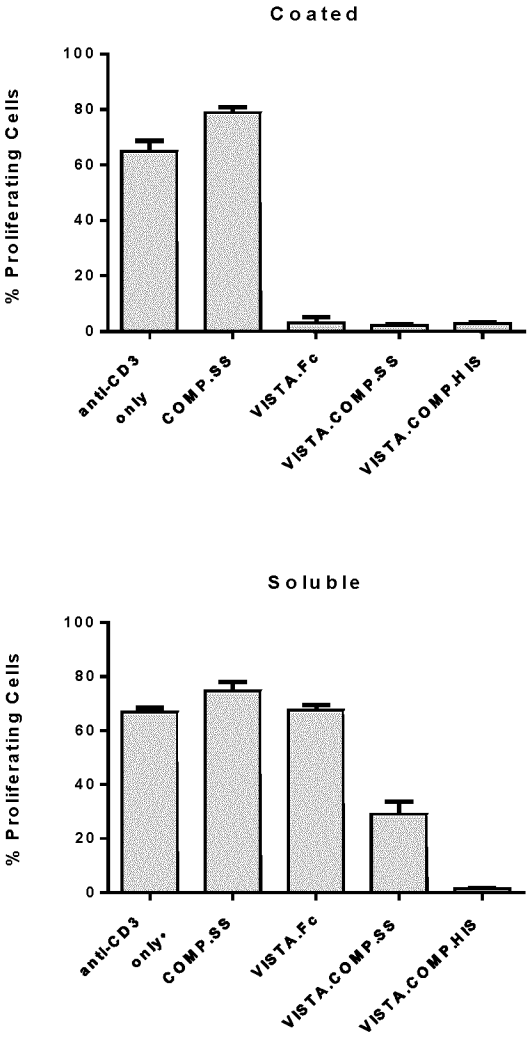


FIGURE 3 A-E

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A



B

	Anti-VISTA mAb clone MH5a	Anti-VISTA mAb clone 730802
VISTA.COMP.his	+	+
VISTA.COMP.SS	+	-

Figure 4 A-B

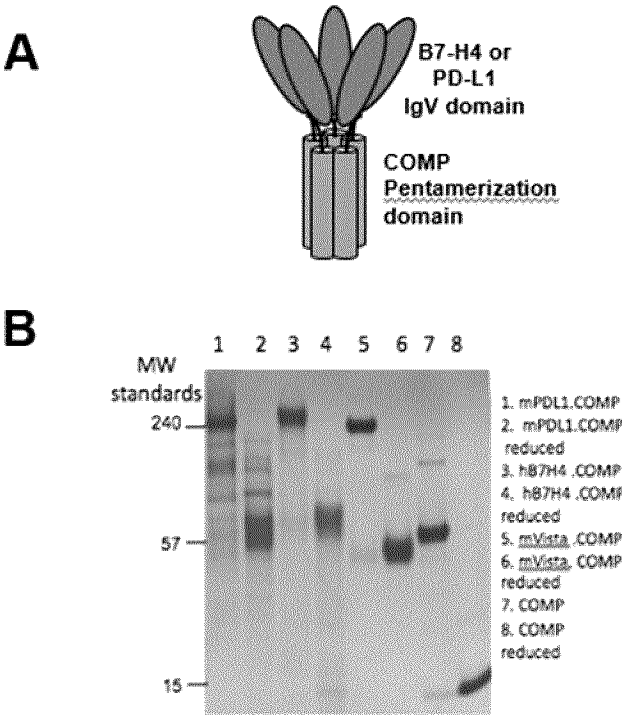


Figure 5 A-B

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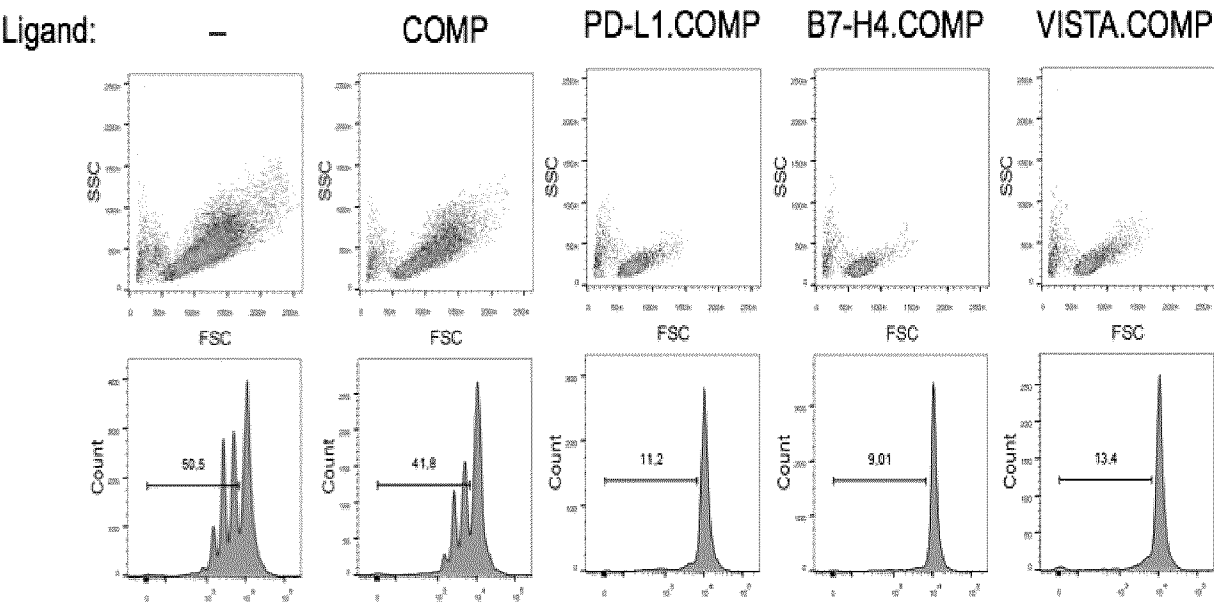


Figure 6

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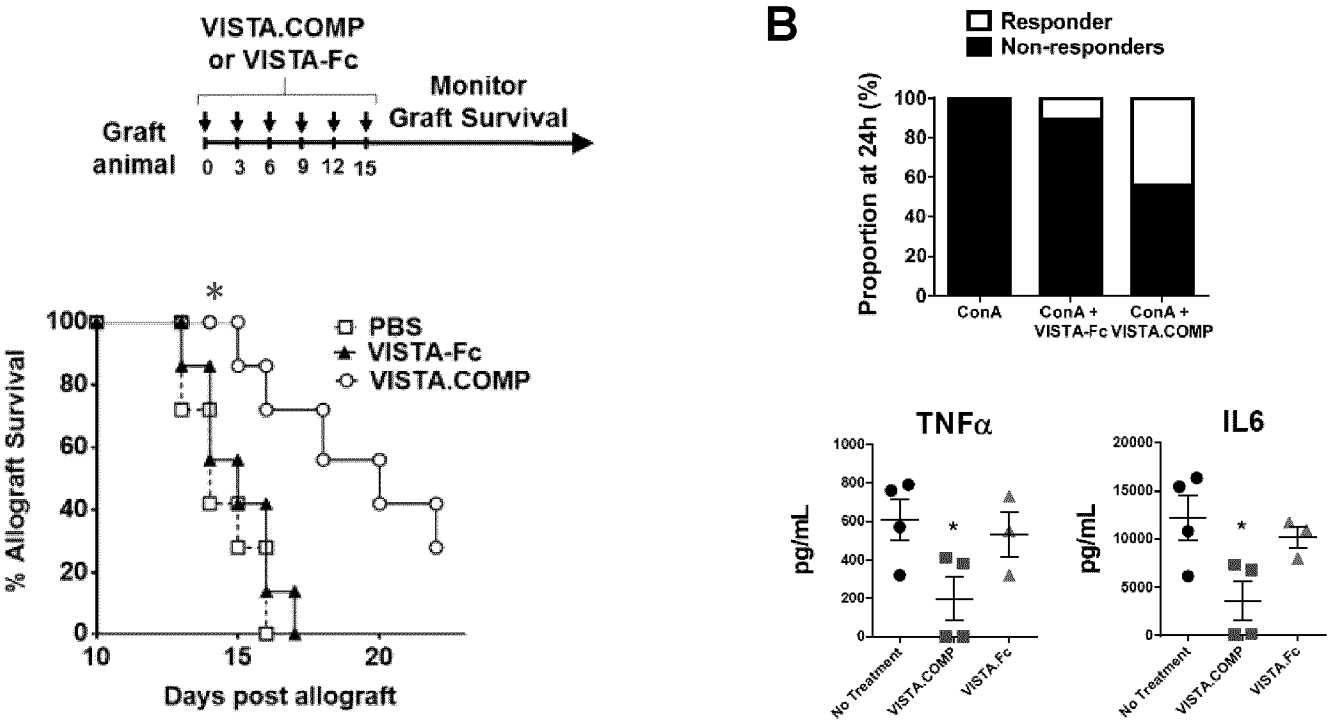


Figure 7 A-B

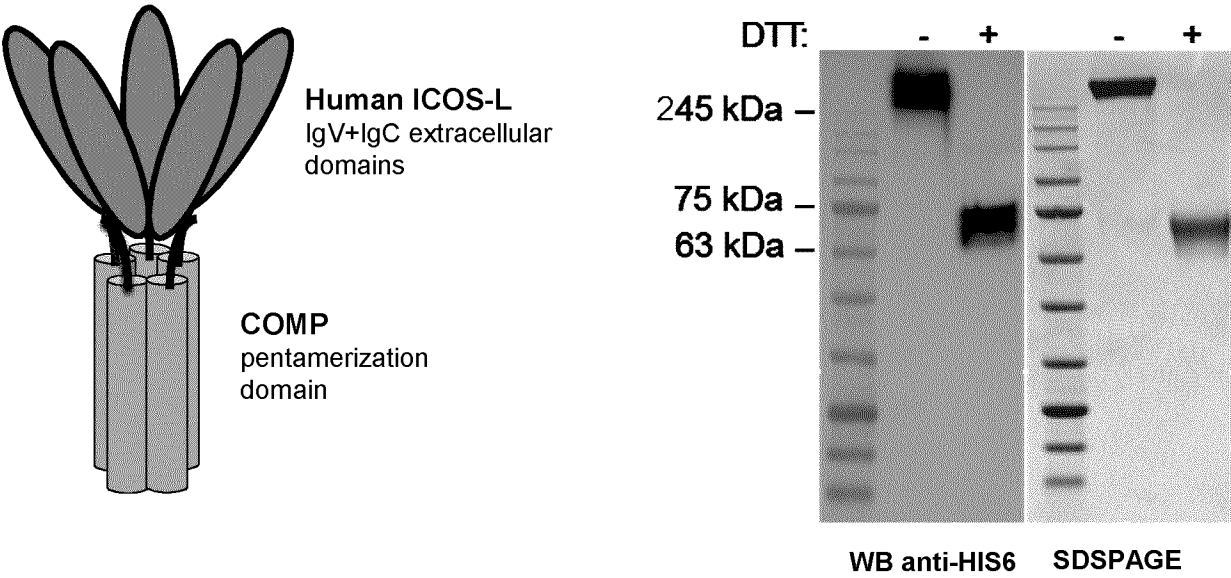


Figure 8 A-B

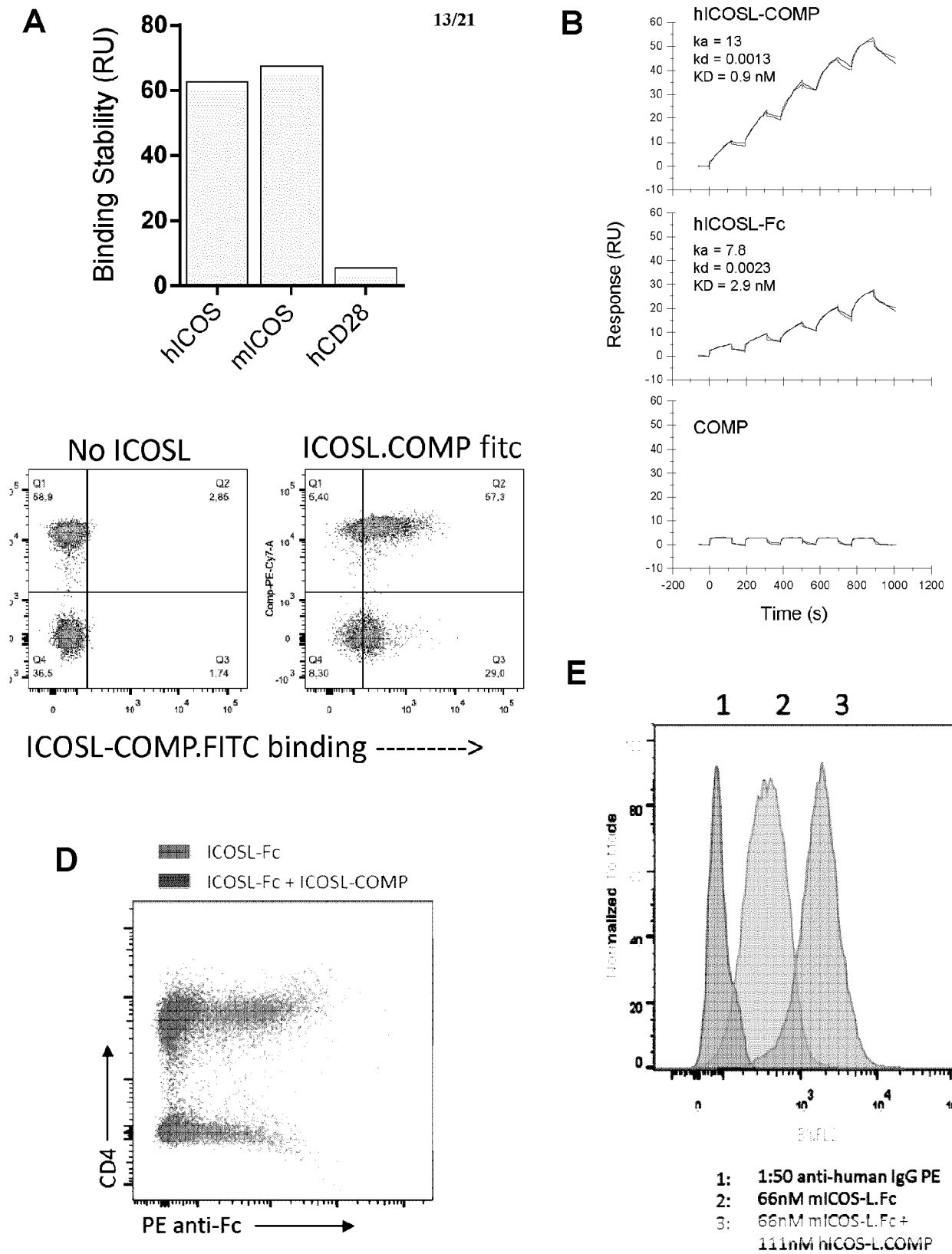
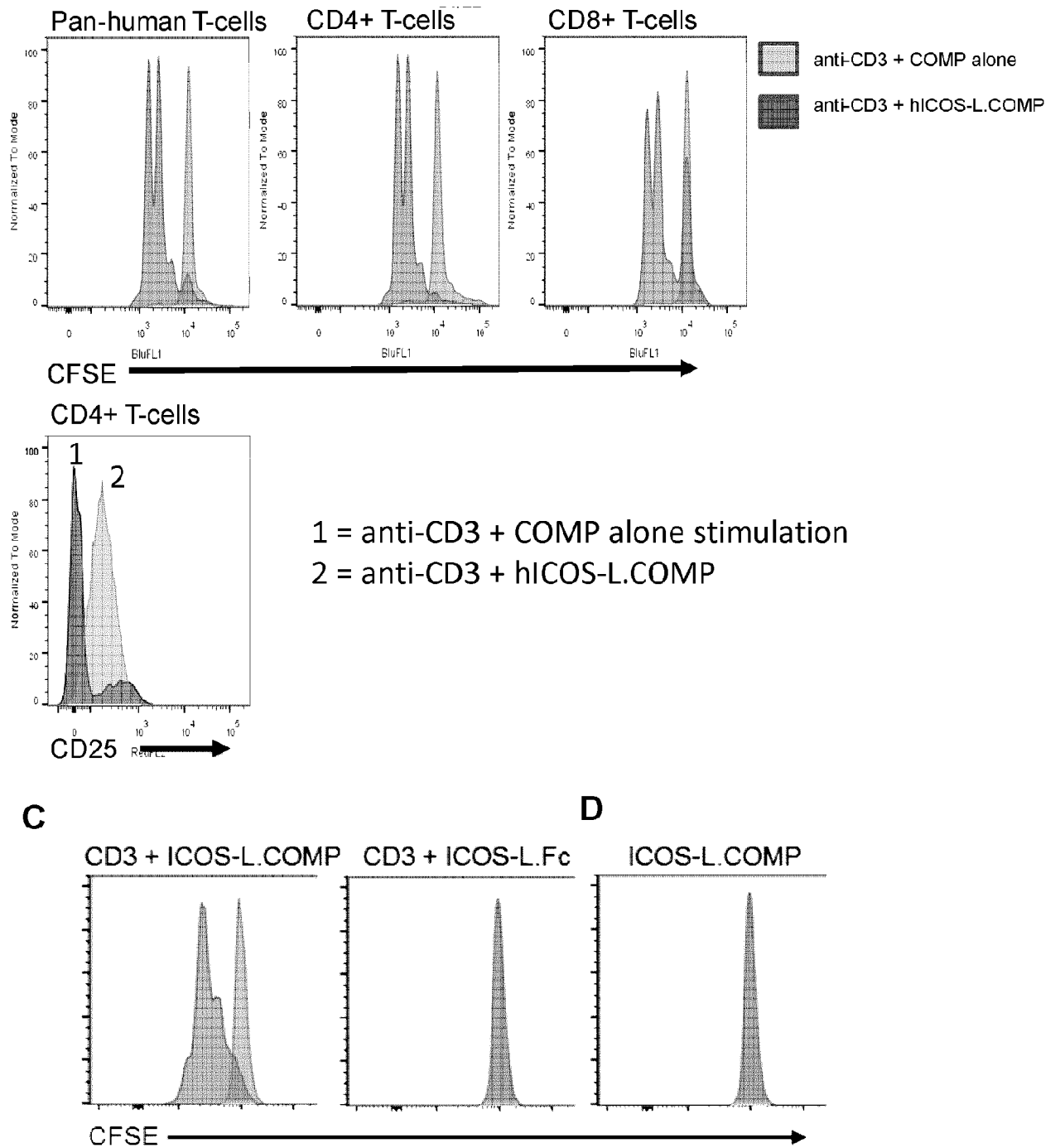


Figure 9 A-E

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**Figure 10 A-D**

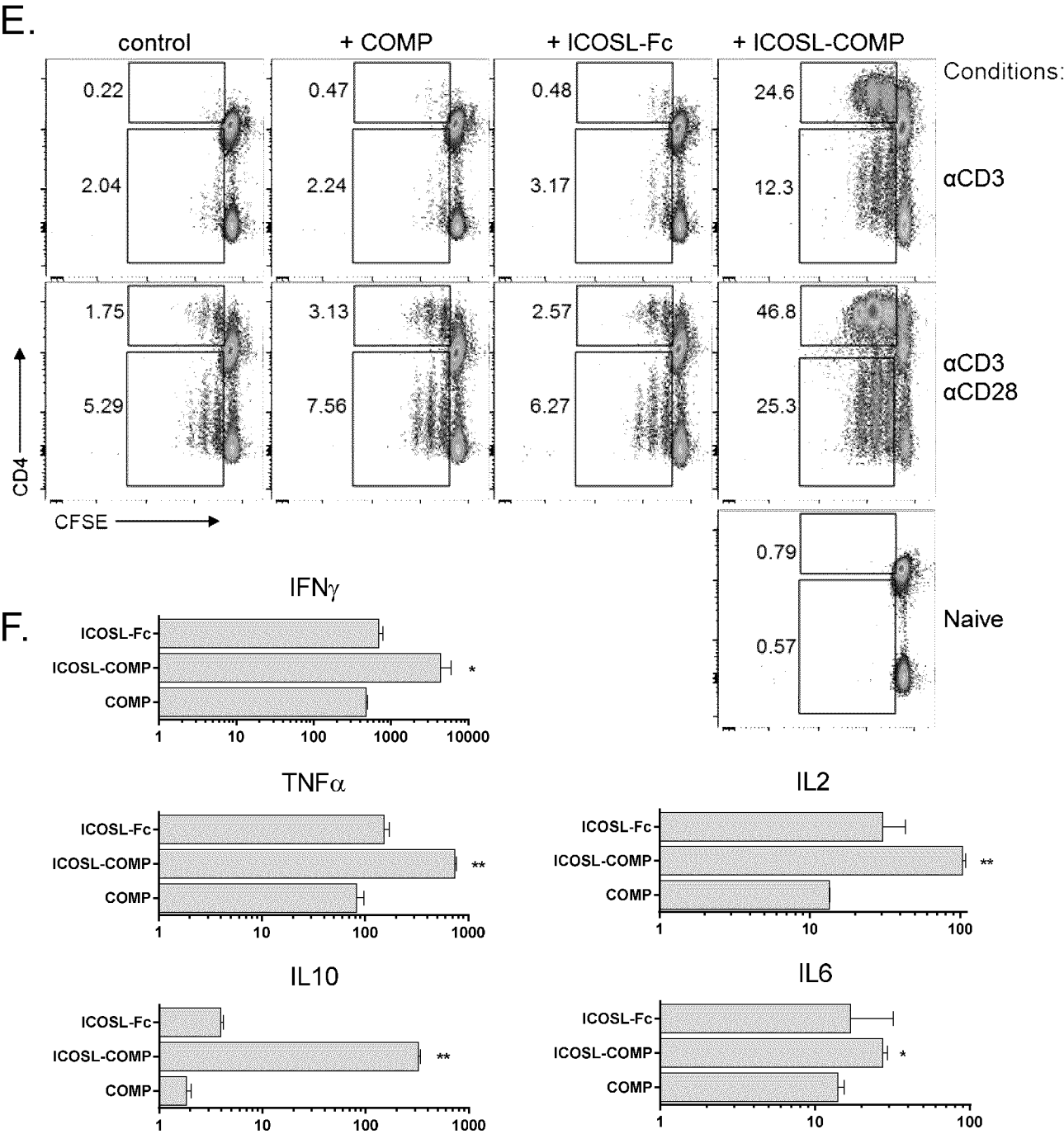


Figure 10 E-F

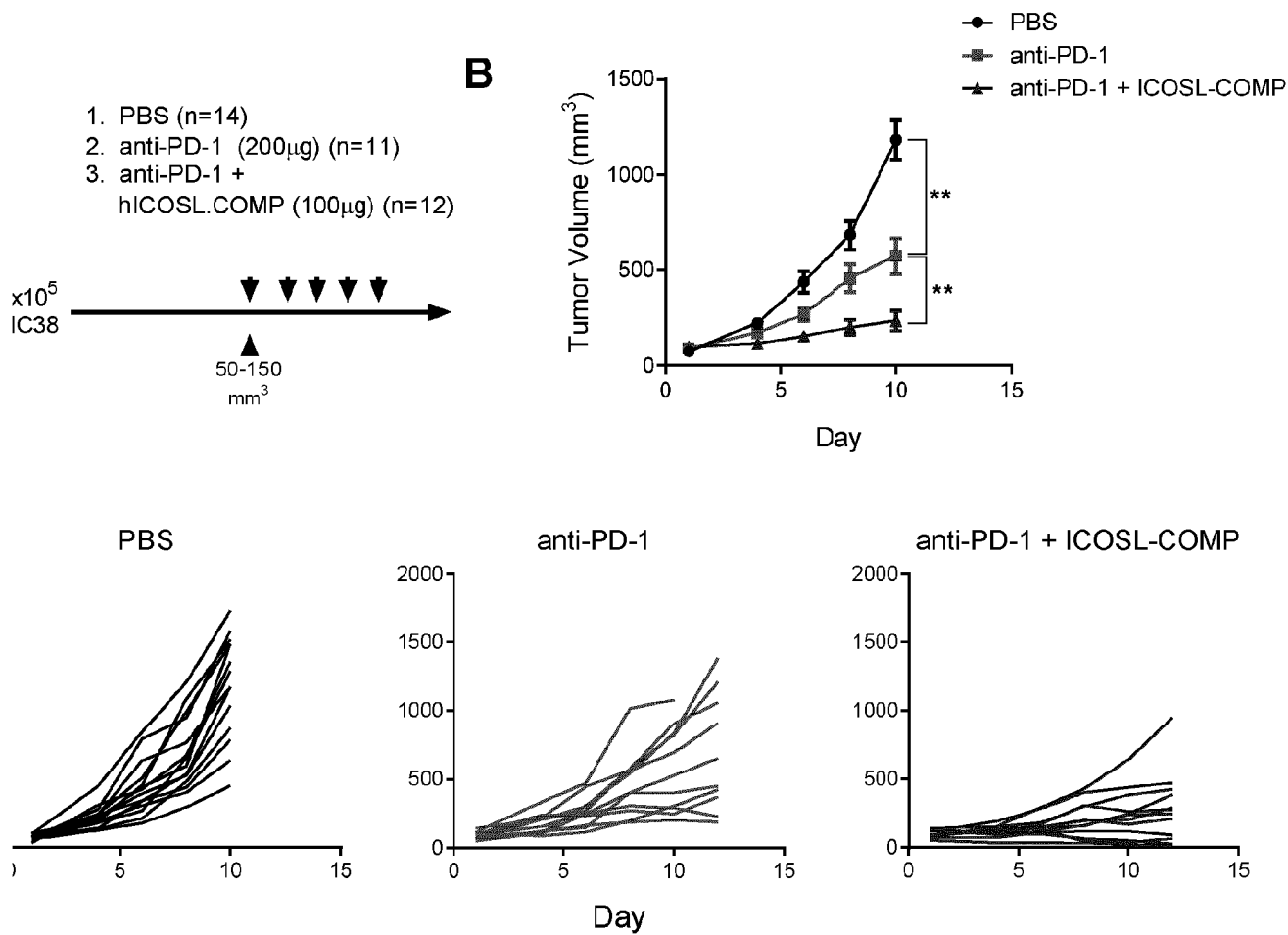


Figure 11 A-C

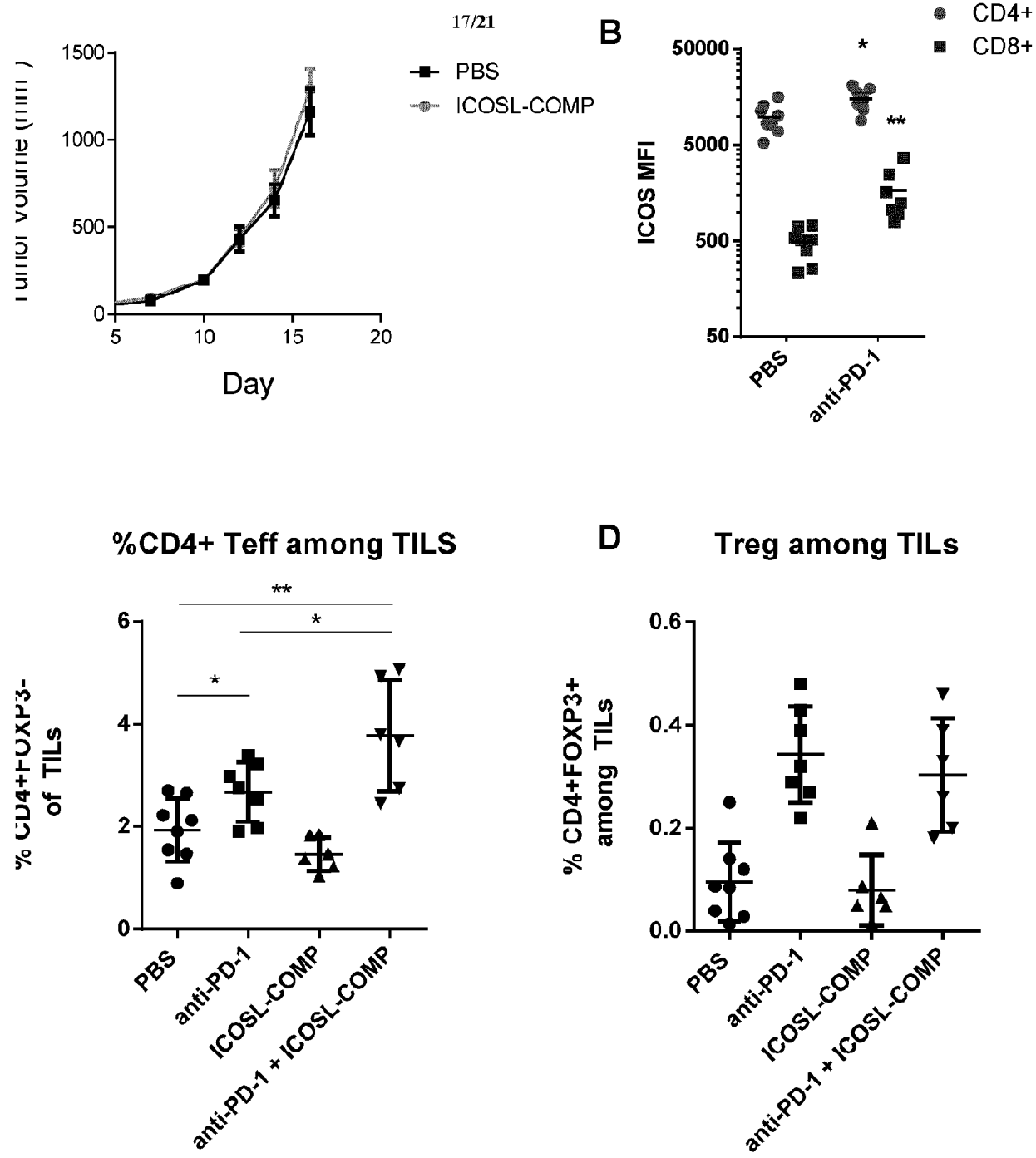


Figure 12 A-D

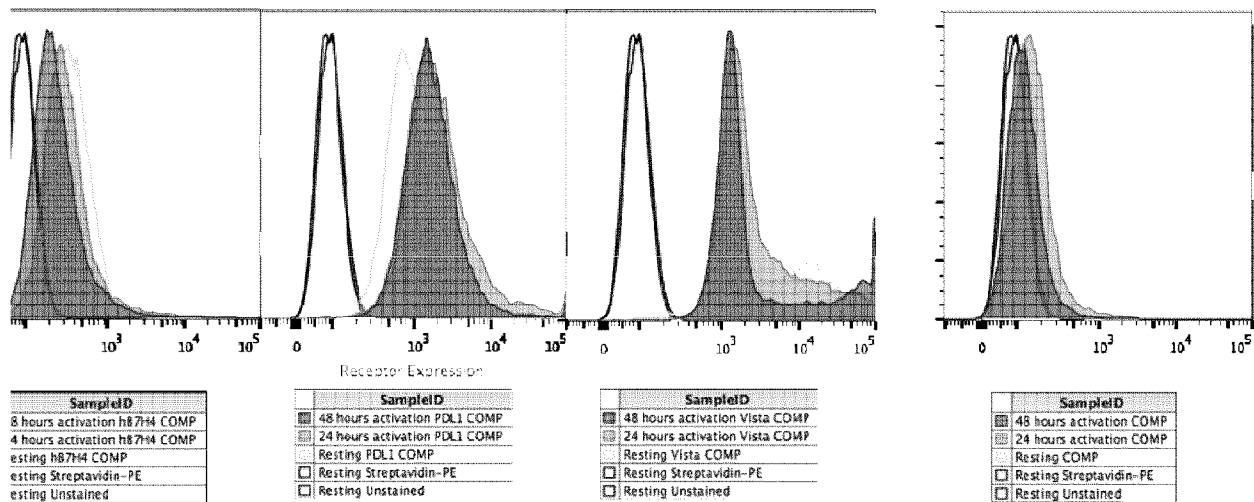


Figure 13

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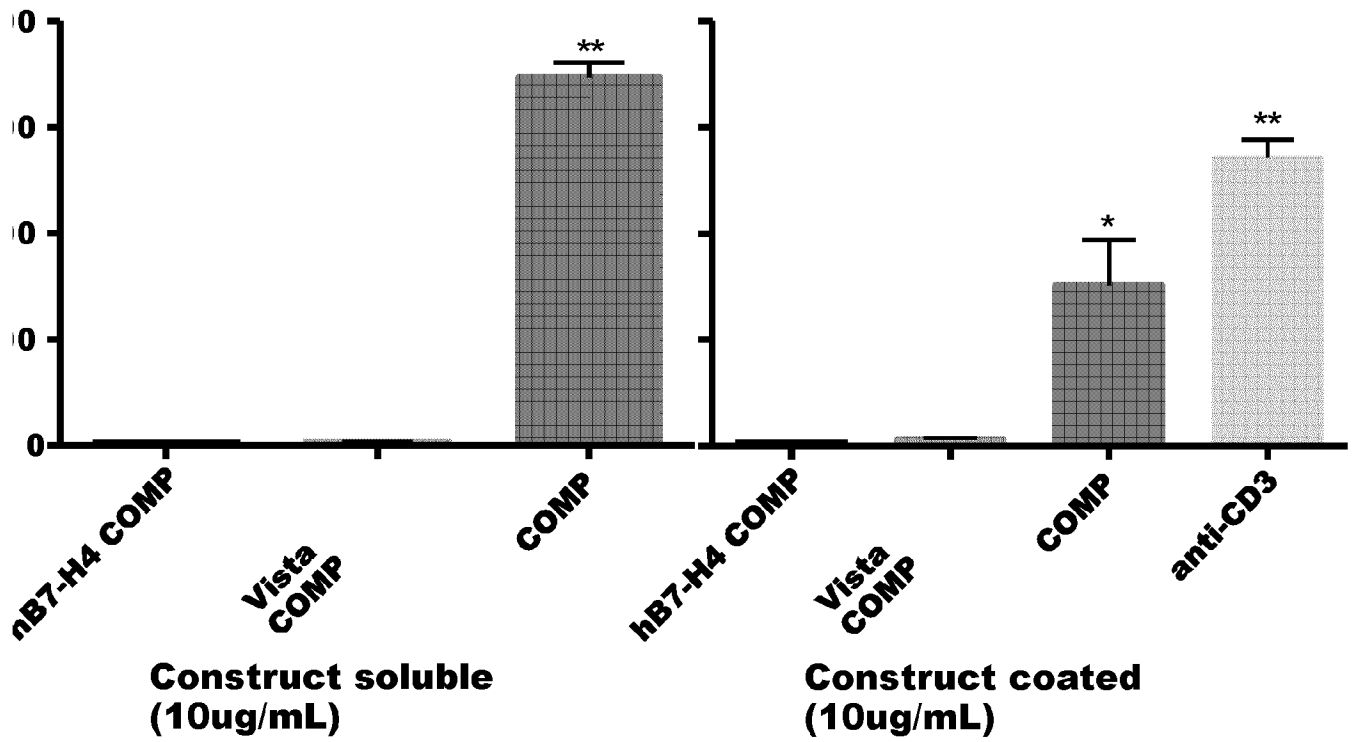


Figure 14

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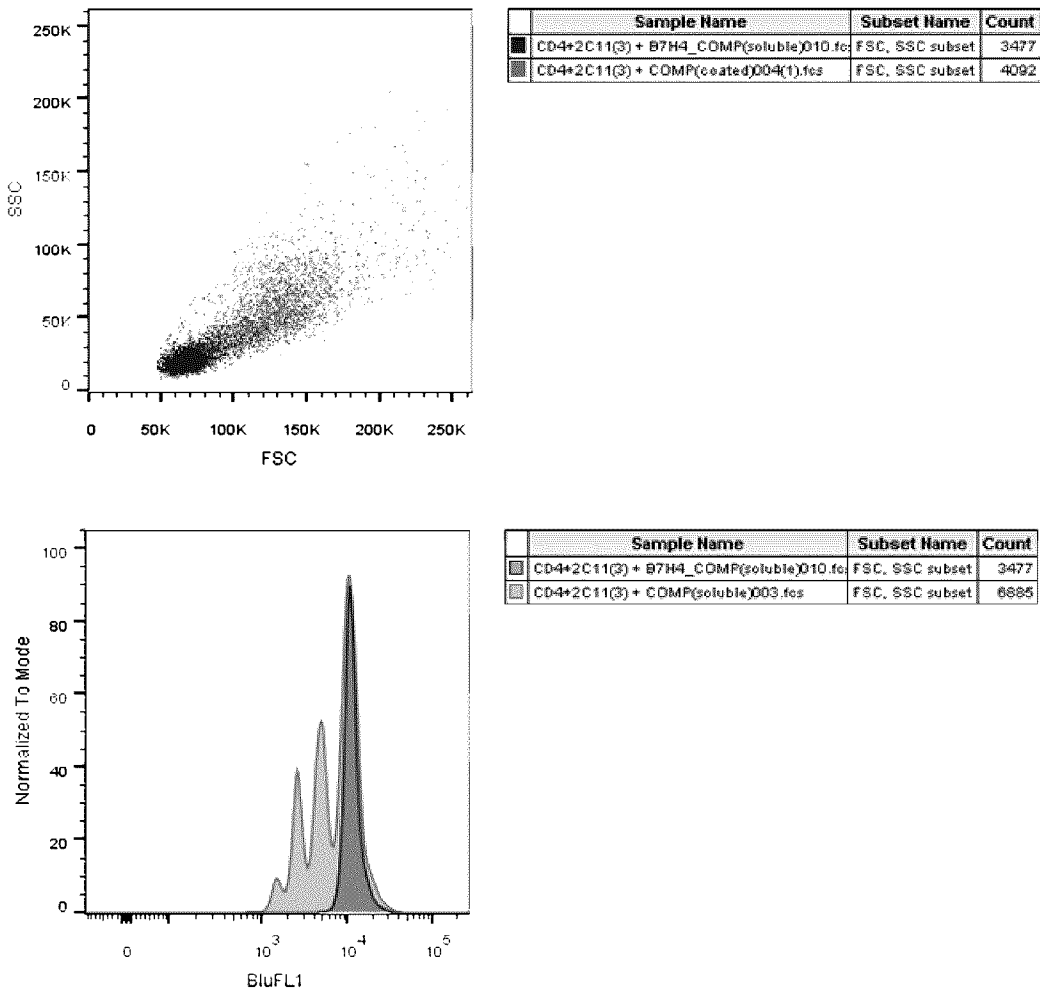


Figure 15

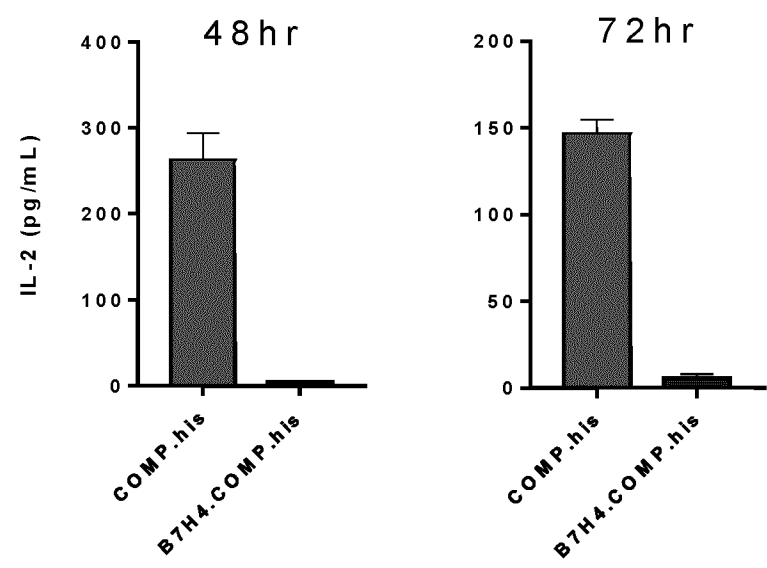


Figure 16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2018/050382

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C12N 15/62** (2006.01), **A61K 39/00** (2006.01), **A61P 35/00** (2006.01), **A61P 37/06** (2006.01), **C07K 14/47** (2006.01)
C07K 14/705 (2006.01), **C07K 14/725** (2006.01), **C07K 14/78** (2006.01), **C07K 19/00** (2006.01), **C12N 5/10** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: **C12N 15/62** (2006.01), **A61K 39/00** (2006.01), **A61P 35/00** (2006.01), **A61P 37/06** (2006.01), **C07K 14/47** (2006.01)
C07K 14/705 (2006.01), **C07K 14/725** (2006.01), **C07K 14/78** (2006.01), **C07K 19/00** (2006.01), **C12N 5/10** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Scopus, Pubmed, Questel Orbit, Genome Quest, Google, Canadian Patent Database (COMP, pentamerization, penta, recombinant, polypeptide, SED ID NO: 11, SEQ ID NO: 49, SEQ ID NO: 9, SEQ ID NO:25, SEQ ID NO:36)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KR20110089015A (NATIONAL CANCER CENTER) 4 August, 2011 (04-08-2011).	1-51
X	WO 2016/151315 A1 (UCL BUSINESS PLC) 29 September, 2016 (29-09-2016). (See pages 4, 9, 16-19 and Figs 5, 6, 7, 10).	1-51
A	WO 98/18943 A1 (NOVATIS AG ET AL.) 7 May, 1998 (07-05-1998).	1-51
A	KIM, H.-Z. ET AL. "A designed angiopoietin-2 variant, pentameric COMP-Ang2, strongly activates Tie2 receptor and stimulates angiogenesis." Biochimica et Biophysica Acta., 772-780., vol. 1793, 2009, pages 772-780, ISSN: 0304-4165.	1-51

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
04-June 2018 (04-06-2018)

Date of mailing of the international search report
06 June 2018 (06-06-2018)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Seema Bissoon-Haqqani (819) 639-7753

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2018/050382

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CLEMENT, G. ET AL. "Peptabodies as tools to test ligands isolated from phage-displayed peptide libraries." Journal of Immunological Methods., vol. 276, 2003, pages 135– 141, ISSN: 0022-1759.	1-51
A	BHATTARAI, G. ET AL. "COMP-Ang1 prevents periodontitic damages and enhances mandible bone growth in an experimental animal model." Bone., vol. 92, 2016, pages 168-179, ISSN: 8756-3282.	1-51
A	ARAKARA, T. ET AL. "Tricomponent fusion complex comprising a viral antigen, apentameric -helical coiled-coil, and an immunoglobulin-bindingdomain as an effective antiviral vaccine." Vaccine., vol. 32, 2014, pages 864-871, ISSN: 0264-410X.	1-51

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Group A: claims 1-14 directed to pentamerized polypeptides; recombinant polypeptides; recombinant nucleic acids; expression vectors, host cells, pharmaceutical compositions and methods based on the extracellular domain of ICOS-L and the pentamerization domain of COMP.

Group B: claims 15-29 directed to pentamerized polypeptides; recombinant polypeptides; recombinant nucleic acids; expression vectors, host cells, pharmaceutical compositions and methods based on the extracellular domain of VISTA and the pentamerization domain of COMP.

Group C: claims 30-40 directed to pentamerized polypeptides; recombinant polypeptides; recombinant nucleic acids; expression vectors, host cells, pharmaceutical compositions and methods based on the extracellular domain of B7-H4 and the pentamerization domain of COMP.

Continued on extra sheet at back

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box No. III

Group D: claims 41-51 directed to pentamerized polypeptides; recombinant polypeptides; recombinant nucleic acids; expression vectors, host cells, pharmaceutical compositions and methods based on the extracellular domain of PD-L1 and the pentamerization domain of COMP.

The common concept linking all the subject matter of claims 1-51 is concept of linking an extracellular domain of a known polypeptide ligand with the pentamerization domain of COMP, which is known in the art (D1 or D2). As such, the subject matter of claims 1-51 lack unity and is instead directed to four separate inventions as outlined in Groups A-D.

However, since all the subject matter of the four Groups A-D could be searched with minimal extra effort, the search and examination was conducted on all subject matter of all the four Groups A-D.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2018/050382

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
KR20110089015A	4 August, 2011 (04-08-2011)	N/A	None
WO2016151315A1	29 September 2016 (29-09-2016)	WO2016151315A1 AU2016238583A1 BR112017018252A2 CA2978852A1 CN107406518A EP3274366A1 GB201504840D0 IL254254D0 JP2018510639A KR20180009738A SG11201706940QA US2018050065A1	29 September 2016 (29-09-2016) 28 September 2017 (28-09-2017) 10 April 2018 (10-04-2018) 29 September 2016 (29-09-2016) 28 November 2017 (28-11-2017) 31 January 2018 (31-01-2018) 06 May 2015 (06-05-2015) 31 October 2017 (31-10-2017) 19 April 2018 (19-04-2018) 29 January 2018 (29-01-2018) 28 September 2017 (28-09-2017) 22 February 2018 (22-02-2018)
WO9818943A1	07 May 1998 (07-05-1998)	WO9818943A1 AT394492T AU6909298A DE69738670D1 EP0938571A1 EP0938571B1 EP0938571B8 ES2309999T3 US2010105876A1	07 May 1998 (07-05-1998) 15 May 2008 (15-05-2008) 22 May 1998 (22-05-1998) 19 June 2008 (19-06-2008) 01 September 1999 (01-09-1999) 07 May 2008 (07-05-2008) 02 July 2008 (02-07-2008) 16 December 2008 (16-12-2008) 29 April 2010 (29-04-2010)
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