

ANTIGEN BINDING PROTEINS BINDING TO 5T4 AND 4-1BB AND RELATED COMPOSITIONS AND METHODS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US Provisional Application Nos. 62/535,107, filed on July 20, 2017; 62/575,820, filed on October 23, 2017; and 62/648,072, filed on March 26, 2018, the contents of which are each incorporated herein by reference in their entireties.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety. A computer readable format copy of the Sequence Listing (filename: APVO_057_03WO_SeqList_ST25.txt, date recorded: July 20, 2018, file size: 260 kilobytes).

FIELD OF THE DISCLOSURE

[0003] The present disclosure relates to molecules that specifically bind to trophoblast glycoprotein (5T4). The molecules may have at least one humanized 5T4-binding domain. These molecules are useful for the characterization or treatment of disorders characterized by expression of 5T4, such as cancer. A protein therapeutic binding to 5T4 may be a monospecific protein therapeutic or a multispecific protein therapeutic. The disclosure also relates to protein therapeutics that specifically bind to tumor necrosis factor receptor superfamily member 9 (4-1BB or CD137). A multispecific protein therapeutic may bind both 5T4-expressing cells and 4-1BB expressed on effector cells to enhance effector cell activation, proliferation, and/or effector-cell mediated cytotoxicity.

BACKGROUND OF THE DISCLOSURE

[0004] 5T4 (also designated trophoblast glycoprotein, TPBG, M6P1 and Waif1) is a well-defined tumor-associated antigen (TAA) originally identified by Professor Peter Stern, University of Manchester (Hole and Stern, 1988). It is an oncofetal antigen expressed in a high proportion of patients in a variety of malignancies, including non-small cell lung, renal, pancreas,

prostate, breast, colorectal, gastric, ovarian and cervix cancers as well as in acute lymphocytic leukemia , and has also been shown to be expressed in tumor-initiating cells (Castro *et al.*, 2012; Damelin *et al.*, 2011; Elkord *et al.*, 2009; Southall *et al.*, 1990).

[0005] Although low levels of 5T4 expression have been detected in some healthy tissue, such as the placenta and specialized epithelia, expression levels in tumors are considerably higher.

[0006] Data suggest that 5T4 regulates the functional activity of CXCR4 (Castro *et al.*, 2012; Southgate *et al.*, 2010). 5T4 binding antibodies or 5T4 knock-down resulted in inhibition of CXCR4-mediated cellular migration, a pathway involved in tumor growth and metastasis. Therefore, targeting 5T4 may provide therapeutic benefits in the treatment of various cancers. Currently there are no FDA approved therapeutics that specifically target or bind 5T4. There is a need for new therapeutics to treat malignancies in which 5T4 is expressed.

[0007] 4-1BB (also known as CD137 or TNFRSF9) is a tumor necrosis factor (TNF) receptor (TNFR) superfamily member. 4-1BB is expressed on various cell populations including activated CD4⁺ and CD8⁺ T cells, regulatory T cells (Treg), dendritic cells (DC), monocytes, mast cells, eosinophils and tumor endothelial cells. Activation of 4-1BB is dependent on receptor oligomerization (Rabu *et al.*, 2005; Wyzgol *et al.*, 2009) induced by binding to 4-1BBL (also known as CD137L), which is expressed as a trimer on the cell surface of antigen presenting cells (APCs) and other cell types. 4-1BB activation on CD8⁺ T cells sustains and augments CD8⁺ T cell effector functions and preferentially supports Th1 cytokine production (Shuford *et al.*, 1997; Lee *et al.*, 2002; Pulle *et al.*, 2006). 4-1BB activation on CD4⁺ T cells, 4-1BB stimulation initially results in activation and later in activation-induced cell death, which is thought to explain why 4-1BB agonistic antibodies have shown therapeutic effect in tumor immunity as well as in autoimmunity (Zhang, JCI, 2007; Sun, Trends Mol Med, 2003). 4-1BB activation has also been reported to suppress Treg function or convert Tregs to cytotoxic CD4⁺ T-cells (Akhmetzyanova *et al.*, 2016; So *et al.*, 2008).

[0008] In addition to expression on and modulation of T cell effector function, 4-1BB is upregulated on CD16- and cytokine-activated natural killer (NK) cells. Activation of 4-1BB has been shown to increase antibody-dependent cellular cytotoxicity (ADCC) activity of NK cells in both murine and human cells (Kohrt 2012 and 2014 J Clin Invest, reviewed by Hout 2012,

Oncoimm). Further, activation of 4-1BB expressed on APCs, such as DCs and macrophages may also induce and/or modulate immune activation.

[0009] The role of 4-1BB in the modulation of immune cell activation suggests that it may be a desirable immunotherapy target in the treatment of multiple cancer types. Indeed, two 4-1BB antibodies are in clinical development: Urelumab (BMS-66513) developed by Bristol-Myers Squibb and PF-05082566 developed by Pfizer. Phase I and II studies in various indications are ongoing for each of the antibodies. However, liver and skin toxicities have been observed in patients and murine models upon 4-1BB activation (Ascierto *et al.*, 2010; Dubrot *et al.*, 2010; Niu *et al.*, 2007). Further, a Phase II study with Urelumab as a second line therapy in metastatic melanoma was terminated in 2009 due to liver toxicity (Garber *et al.*, 2011; Li and Liu, 2013).

[0010] Therefore, there remains a need in the art for therapeutics that safely and effectively activate 4-1BB for use in the treatment of oncologic indications.

SUMMARY OF THE DISCLOSURE

[0011] In some aspects, the disclosure relates to multispecific polypeptides that specifically bind to 5T4 and/or 4-1BB. In some embodiments, a 5T4-binding domain of the disclosure binds to an extracellular domain of human 5T4. In certain embodiments, a 4-1BB-binding domain of the disclosure binds to an extracellular domain of human 4-1BB. In some embodiments, the disclosure provides a multispecific polypeptide comprising a 5T4-binding domain that specifically binds to human 5T4 and a 4-1BB-binding domain, wherein said 5T4-binding domain comprises: (i) an immunoglobulin heavy chain variable region comprising HCDR1, HCDR2, and HCDR3, and (ii) an immunoglobulin light chain variable region comprising LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises an amino acid sequence of SEQ ID NO: 30; (b) the HCDR2 comprises an amino acid sequence of SEQ ID NO: 32; (c) the HCDR3 comprises an amino acid sequence of SEQ ID NO: 34; (d) the LCDR1 comprises an amino acid sequence of SEQ ID NO: 42; (e) the LCDR2 comprises an amino acid sequence of SEQ ID NO: 10; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 36. In some embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to a sequence selected from the group consisting of SEQ ID NOs: 38 and 46. In certain embodiments, the 5T4-binding domain comprises a heavy chain variable region

comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 and 46. In other embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to a sequence selected from the group consisting of SEQ ID NOs: 44, 48, and 50. In some embodiments, the 5T4-binding domain comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 48, and 50. In other embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises i) a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 38 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 44; or ii) a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 48; or iii) a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 5. In some embodiments, the 5T4-binding domain comprises i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 38 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 44; or ii) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 48; or iii) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 50.

[0012] In certain aspects, the disclosure includes a multispecific polypeptide comprising a 5T4-binding domain that specifically binds to human 5T4 and a 4-1BB-binding domain that specifically binds to human 4-1BB, wherein each of the 4-1BB-binding domain and 5T4 binding domain comprise (i) an immunoglobulin heavy chain variable region (VH) comprising HCDR1, HCDR2, and HCDR3; and (ii) an immunoglobulin light chain variable region (VL) comprising LCDR1, LCDR2, and LCDR3, wherein the VH and/or the VL region of the 5T4-binding domain comprise one or more mutations in the framework region; and wherein the 5T4-binding domain comprises (a) the HCDR1 comprising an amino acid sequence of SEQ ID NO: 30;

(b) the HCDR2 comprising an amino acid sequence of SEQ ID NO: 32; (c) the HCDR3 comprising an amino acid sequence of SEQ ID NO: 34; (d) the LCDR1 comprising an amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprising an amino acid sequence of SEQ ID NO: 10; and (f) the LCDR3 comprising an amino acid sequence of SEQ ID NO: 36. In some embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NOs: 38. In one embodiment, the 5T4-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 38. In some embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 40. In certain embodiments, the 5T4-binding domain comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO: 40. In some embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 38 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 40. In some embodiments, 5T4-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 38 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 40.

[0013] The disclosure further provides a multispecific polypeptide comprising a 5T4-binding domain that specifically binds to human 5T4 and a 4-1BB-binding domain that specifically binds to human 4-1BB, wherein each of the 4-1BB-binding domain and 5T4 binding domain comprise (i) an immunoglobulin heavy chain variable region (VH) comprising HCDR1, HCDR2, and HCDR3; and (ii) an immunoglobulin light chain variable region (VL) comprising LCDR1, LCDR2, and LCDR3, wherein the VH and/or the VL region of the 4-1BB-binding domain comprise one or more mutations in the framework region; and wherein the 5T4-binding domain comprises (a) the HCDR1 comprising an amino acid sequence of SEQ ID NO: 30; (b) the HCDR2 comprising an amino acid sequence of SEQ ID NO: 32; (c) the HCDR3 comprising an amino acid sequence of SEQ ID NO: 34; (d) the LCDR1 comprising an amino acid sequence of SEQ ID NO: 42; (e) the LCDR2 comprising an amino acid sequence of SEQ ID NO: 10; and (f) the LCDR3 comprising an amino acid sequence of SEQ ID NO: 36. In some embodiments, the 5T4-binding

domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to a sequence selected from the group consisting of SEQ ID NOs: 38 and 46. In certain embodiments, the 5T4-binding domain comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 and 46. In some embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to a sequence selected from the group consisting of SEQ ID NOs: 44, 48, and 50. In some aspects, the 5T4-binding domain comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 48, and 50. In certain embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises i) a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 38 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 44; or ii) a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 48; or iii) a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 50. In some embodiments, the 5T4-binding domain comprises i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 38 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 44; or ii) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 48; or iii) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 50.

[0014] In some aspects, the disclosure provides a multispecific polypeptide comprising a 5T4-binding domain that specifically binds to human 5T4 and a 4-1BB-binding domain that specifically binds to human 4-1BB, wherein each of the 4-1BB-binding domain and 5T4 binding domain comprise (i) an immunoglobulin heavy chain variable region (VH)

comprising HCDR1, HCDR2, and HCDR3; and (ii) an immunoglobulin light chain variable region (VL) comprising LCDR1, LCDR2, and LCDR3, wherein the VH and/or the VL region of the 4-1BB-binding domain comprise one or more mutations in the framework region; and wherein the 5T4-binding domain comprises (a) the HCDR1 comprising an amino acid sequence of SEQ ID NO: 52; (b) the HCDR2 comprising an amino acid sequence of SEQ ID NO: 32; (c) the HCDR3 comprising an amino acid sequence of SEQ ID NO: 34; (d) the LCDR1 comprising an amino acid sequence of SEQ ID NO: 54; (e) the LCDR2 comprising an amino acid sequence of SEQ ID NO: 10; and (f) the LCDR3 comprising an amino acid sequence of SEQ ID NO: 36. In certain embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 56. In some aspects, the 5T4-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 56. In certain embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NOs: 58. In some embodiments, the 5T4-binding domain comprises a light chain variable region comprising an amino acid sequence of SEQ ID NOs: 58. In certain embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 56 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 58. In some embodiments, the 5T4-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 56 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58.

[0015] The disclosure also provides a multispecific polypeptide comprising a 5T4-binding domain that specifically binds to human 5T4 and a 4-1BB-binding domain that specifically binds to human 4-1BB, wherein each of the 4-1BB-binding domain and 5T4 binding domain comprise (i) an immunoglobulin heavy chain variable region (VH) comprising HCDR1, HCDR2, and HCDR3; and (ii) an immunoglobulin light chain variable region (VL) comprising LCDR1, LCDR2, and LCDR3, wherein the VH and/or the VL region of the 4-1BB-binding domain comprise one or more mutations in the framework region; and wherein the 5T4-binding domain comprises (a) the HCDR1 comprising an amino acid sequence of SEQ ID NO: 60; (b) the HCDR2

comprising an amino acid sequence of SEQ ID NO: 62; (c) the HCDR3 comprising an amino acid sequence of SEQ ID NO: 34; (d) the LCDR1 comprising an amino acid sequence of SEQ ID NO: 54; (e) the LCDR2 comprising an amino acid sequence of SEQ ID NO: 10; and (f) the LCDR3 comprising an amino acid sequence of SEQ ID NO: 36. In certain embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 64. In some embodiments, the 5T4-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 64. In certain aspects, the 5T4-binding domain comprising the CDR sequences recited above comprises a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NOs: 58. In some embodiments, the 5T4-binding domain comprises a light chain variable region comprising an amino acid sequence of SEQ ID NOs: 58. In certain embodiments, the 5T4-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 64 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 58. In some embodiments, the 5T4-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 64 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58.

[0016] In certain aspects, the disclosure provides a multispecific polypeptide comprising a 4-1BB-binding domain that specifically binds to human 4-1BB. The disclosure also provides a multispecific polypeptide comprising a 4-1BB-binding domain and a 5T4-binding domain, wherein the 4-1BB-binding domain is linked to the 5T4-binding domain via a binding domain linker. In some embodiments, a multispecific polypeptide comprises, from amino-terminus to carboxyl-terminus, (i) a 5T4-binding domain, (ii) a binding domain linker, and (iii) a 4-1BB-binding domain. In certain aspects, the 5T4-binding domain is a single chain variable fragment (scFv). In some embodiments, the light chain variable region of said scFv is carboxy-terminal to the heavy chain variable region of said scFv. In other embodiments, the light chain variable region of said scFv is amino-terminal to the heavy chain variable region of said scFv. In certain aspects, the scFv comprises a linker polypeptide. In some examples, the linker polypeptide is between the light chain variable region and the heavy chain variable region of said scFv. In

certain embodiments, the linker polypeptide comprises a Gly₄Ser linker. In some embodiments, the linker polypeptide comprises the formula (Gly₄Ser)_n, wherein n = 1-5. In some aspects, the linker polypeptide comprises an amino acid sequence selected from SEQ ID NOs: 85-108. In certain embodiments, the linker polypeptide comprises an amino acid sequence set forth in SEQ ID NO: 98 (GGGGSGGGSGGGSGGGGS).

[0017] In certain embodiments, an scFv comprising the CDR sequences recited above comprises a sequence having at least 80%, at least 85%, at least 90%, or at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 118, 120, 122, 124, 126, 128, 130, 132, 134, and 170. In some embodiments, an scFv comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 118, 120, 122, 124, 126, 128, 130, 132, 134, and 170.

[0018] In some aspects, the disclosure provides a multispecific polypeptide comprising a 4-1BB-binding domain that specifically binds to human 4-1BB and a 5T4-binding domain, wherein said 4-1BB-binding domain comprises: (i) an immunoglobulin heavy chain variable region (VH) comprising HCDR1, HCDR2, and HCDR3, and (ii) an immunoglobulin light chain variable region (VL) comprising LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises an amino acid sequence of SEQ ID NO: 2; (b) the HCDR2 comprises an amino acid sequence of SEQ ID NO: 4; (c) the HCDR3 comprises an amino acid sequence of SEQ ID NO: 6; (d) the LCDR1 comprises an amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises an amino acid sequence of SEQ ID NO: 10; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 12. In certain embodiments, the 4-1BB-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 14. In some embodiments, the 4-1BB-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 14. In certain embodiments, the 4-1BB-binding domain comprising the CDR sequences recited above comprises a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 16. In some embodiments, the 4-1BB-binding domain comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO: 16. In certain embodiments, the 4-1BB-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97%

identity to SEQ ID NO: 14 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 16. In some aspects, the 4-1BB-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 14 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 16.

[0019] The disclosure further provides a multispecific polypeptide comprising a 5T4-binding domain that specifically binds to human 5T4 and a 4-1BB-binding domain, wherein the 4-1BB-binding domain comprises (i) an immunoglobulin heavy chain variable region (VH) comprising HCDR1, HCDR2, and HCDR3; and (ii) an immunoglobulin light chain variable region (VL) comprising LCDR1, LCDR2, and LCDR3, and wherein the VH and/or the VL region of the 4-1BB-binding domain comprise one or more mutations in the framework region, and wherein the 4-1BB-binding domain comprises (a) the HCDR1 comprising an amino acid sequence of SEQ ID NO: 18; (b) the HCDR2 comprising an amino acid sequence of SEQ ID NO: 4; (c) the HCDR3 comprising an amino acid sequence of SEQ ID NO: 6; (d) the LCDR1 comprising an amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprising an amino acid sequence of SEQ ID NO: 10; and (f) the LCDR3 comprising an amino acid sequence of SEQ ID NO: 12. In certain embodiments, the 4-1BB-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 20. In some embodiments, the 4-1BB-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 20. In certain embodiments, the 4-1BB-binding domain comprising the CDR sequences recited above comprises a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 22. In some embodiments, the 4-1BB-binding domain comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO: 22. In certain embodiments, the 4-1BB-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 20 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 22. In some embodiments, the 4-1BB-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 20 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 22.

[0020] The disclosure also provides a multispecific polypeptide comprising a 5T4-binding domain that specifically binds to human 5T4 and a 4-1BB-binding domain, wherein the 4-1BB-binding domain comprises (i) an immunoglobulin heavy chain variable region (V_H) comprising HCDR1, HCDR2, and HCDR3; and (ii) an immunoglobulin light chain variable region (V_L) comprising LCDR1, LCDR2, and LCDR3, and wherein the V_H and/or the V_L region of the 4-1BB-binding domain comprise one or more mutations in the framework region, and wherein the 4-1BB-binding domain comprises (a) the HCDR1 comprising an amino acid sequence of SEQ ID NO: 24; (b) the HCDR2 comprising an amino acid sequence of SEQ ID NO: 4; (c) the HCDR3 comprising an amino acid sequence of SEQ ID NO: 6; (d) the LCDR1 comprising an amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprising an amino acid sequence of SEQ ID NO: 10; and (f) the LCDR3 comprising an amino acid sequence of SEQ ID NO: 12. In certain embodiments, the 4-1BB-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 26. In some embodiments, the 4-1BB-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 26. In certain embodiments, the 4-1BB-binding domain comprising the CDR sequences recited above comprises a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 16. In some embodiments, the 4-1BB-binding domain comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO: 16. In certain embodiments, the 4-1BB-binding domain comprising the CDR sequences recited above comprises a heavy chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 26 and a light chain variable region comprising an amino acid sequence having at least 90%, at least 95%, or at least 97% identity to SEQ ID NO: 16. In some embodiments, the 4-1BB-binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 26 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 16.

[0021] In certain embodiments, the disclosure provides a multispecific polypeptide comprising a 4-1BB-binding domain wherein the 4-1BB-binding domain is a single chain variable fragment (scFv). In some aspects, the light chain variable region of said scFv is carboxy-terminal to the heavy chain variable region of said scFv. In other aspects, the light chain variable region of said scFv is amino-terminal to the heavy chain variable region of said scFv. In certain aspects, the

scFv comprises a linker polypeptide. In some examples, the linker polypeptide is between the light chain variable region and the heavy chain variable region of said scFv. In certain embodiments, the linker polypeptide comprises a Gly₄Ser linker. In some embodiments, the linker polypeptide comprises the formula (Gly₄Ser)_n, wherein n = 1-5. In some aspects, the linker polypeptide comprises an amino acid sequence selected from SEQ ID NOs: 85-108. In certain embodiments, the linker polypeptide comprises an amino acid sequence set forth in SEQ ID NO: 98 (GGGGSGGGGSGGGGSGGGGS). In certain aspects, a multispecific polypeptide of the disclosure comprises a 4-1BB-binding domain that is linked to a 5T4-binding domain via a binding domain linker. In some embodiments, a multispecific polypeptide comprises, from amino-terminus to carboxyl-terminus, (i) the 5T4-binding domain, (ii) a binding domain linker, and (iii) the 4-1BB-binding domain.

[0022] In some embodiments, the 4-1BB-binding domain comprising the CDR sequences recited above comprises a sequence having at least 80%, at least 85%, at least 90%, or at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 110, 112, 114, and 116. In certain embodiments, the 4-1BB-binding domain comprises a sequence selected from the group consisting of SEQ ID NOs: 110, 112, 114, and 116.

[0023] In certain aspects, a multispecific polypeptide of the disclosure comprises a 4-1BB-binding domain that is conjugated to a drug or a toxin.

[0024] The disclosure further provides a multispecific polypeptide comprising a 5T4-binding domain that is fused or conjugated to an immunoglobulin constant region. An immunoglobulin constant region may be a human Fc domain. In some embodiments, the human Fc domain comprises a sequence set forth in SEQ ID NO: 158 or SEQ ID NO: 160. In certain embodiments, the disclosure provides a multispecific polypeptide that comprises, from amino-terminus to carboxyl-terminus, (i) a 5T4-binding domain, (ii) a hinge region, (iii) an immunoglobulin constant region, (iv) a binding domain linker, and (v) a 4-1BB-binding domain. In some embodiments, the binding domain linker comprises a Gly₄Ser sequence. In some examples, the binding domain linker comprises the formula (Gly₄Ser)_n, wherein n = 1-5. In some aspects, the binding domain linker comprises an amino acid sequence selected from SEQ ID NOs: 85-108. In certain embodiments, the binding domain linker comprises an amino acid sequence set forth in SEQ ID NO: 107 (SGGGSGGGGSGGGGSPS).

[0025] In some aspects, the disclosure also provides a multispecific polypeptide comprising a first scFv domain and a second scFv domain, wherein the first and second scFv domains are linked together by a binding domain linker or a binding domain linker and an immunoglobulin Fc domain, wherein the immunoglobulin Fc domain comprises a hinge region and an immunoglobulin constant region; and wherein the second scFv domain specifically binds to human 4-1BB and comprises: (i) an immunoglobulin heavy chain variable region comprising an HCDR1 amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 24, an HCDR2 amino acid sequence of SEQ ID NO: 4, and an HCDR3 amino acid sequence of SEQ ID NOs: 6; and (ii) an immunoglobulin light chain variable region comprising an LCDR1 amino acid sequence of SEQ ID NOs: 8, an LCDR2 amino acid sequence of SEQ ID NO: 10, and an LCDR3 amino acid sequence of SEQ ID NO: 12, wherein the first and second scFv domains are linked together by a binding domain linker or a binding domain linker and an immunoglobulin Fc domain, wherein the immunoglobulin Fc domain comprises a hinge region and an immunoglobulin constant region. In certain embodiments, this polypeptide comprises an amino acid sequence comprising at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, and 156. In some aspects, the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, and 156. In some embodiments, the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 136, 138, and 146.

[0026] In some embodiments, the multispecific polypeptide comprises, from amino-terminus to carboxyl-terminus, (i) the first scFv domain, (ii) a binding domain linker, and (iii) the second binding domain. In other embodiments, the multispecific polypeptide comprises, from amino-terminus to carboxyl-terminus, (i) the second scFv domain, (ii) a binding domain linker, and (iii) the first scFv domain. In certain aspects, the multispecific polypeptide comprises, from amino-terminus to carboxyl-terminus, (i) the first scFv domain, (ii) a hinge region, (iii) an immunoglobulin constant region, (iv) a binding domain linker, and (v) the second scFv domain. In some embodiments, the binding domain linker comprises a Gly₄Ser sequence. In some embodiments, the binding domain linker comprises the formula (Gly₄Ser)_n, wherein n = 1-5. In certain aspects, the binding domain linker comprises an amino acid sequence selected from SEQ ID NOs: 85-108. In certain embodiments, the binding domain linker comprises an amino acid

sequence set forth in SEQ ID NO: 107 (SGGGGSGGGGSGGGGSPS). In some embodiments, the second scFv domain of a multispecific polypeptide comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 2; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 4; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 6; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 8; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 12. In other embodiments, the second scFv domain of a multispecific polypeptide comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 24; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 4; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 6; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 8; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 12. In some embodiments, the second scFv domain of a multispecific polypeptide comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 14 and a light chain variable region of SEQ ID NO: 16. In other embodiments, the second scFv domain of a multispecific polypeptide comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 20 and a light chain variable region of SEQ ID NO: 22. In some embodiments, the second scFv domain of a multispecific polypeptide comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 26 and a light chain variable region of SEQ ID NO: 16. In other embodiments, the second scFv domain of a multispecific polypeptide comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 28 and a light chain variable region of SEQ ID NO: 16.

[0027] The disclosure further provides a multispecific polypeptide comprising a first scFv domain and a second scFv domain, wherein the first and second scFv domains are linked together by a binding domain linker or a binding domain linker and an immunoglobulin Fc domain, wherein the immunoglobulin Fc domain comprises a hinge region and an immunoglobulin constant region; wherein the second scFv domain specifically binds to human 4-1BB; and wherein the first scFv domain comprises (i) an immunoglobulin heavy chain variable region comprising an HCDR1 amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 52, and 60, an HCDR2 amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 62, and an HCDR3 amino acid sequence of SEQ ID NO: 34; and (ii) an immunoglobulin light chain variable region comprising an LCDR1 amino acid sequence selected from the group consisting of

SEQ ID NOs: 8, 42, and 54, an LCDR2 amino acid sequence of SEQ ID NOs: 10, and an LCDR3 amino acid sequence of SEQ ID NOs: 36; and wherein the second scFv domain comprises (i) an immunoglobulin heavy chain variable region comprising an HCDR1 amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 24, an HCDR2 amino acid sequence selected of SEQ ID NO: 4, and an HCDR3 amino acid sequence of SEQ ID NO: 6; and (ii) an immunoglobulin light chain variable region comprising an LCDR1 amino acid sequence of SEQ ID NO: 8, an LCDR2 amino acid sequence of SEQ ID NO: 10, and an LCDR3 amino acid sequence of SEQ ID NO: 12, wherein the first and second scFv domains are linked together by a binding domain linker or a binding domain linker and an immunoglobulin Fc domain, wherein the immunoglobulin Fc domain comprises a hinge region and an immunoglobulin constant region. In some embodiments, the second scFv domain comprises (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 2; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 4; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 6; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 8; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 12. In certain embodiments, the first scFv domain comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 30; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 32; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 34; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 8; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 36. In some embodiments, the first scFv domain comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 30; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 32; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 34; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 42; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 36. In certain aspects, the first scFv domain comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 30; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 32; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 34; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 42; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 36. In some embodiments, the first scFv domain comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 52; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 32; (c) the HCDR3 amino acid sequence set forth

in SEQ ID NO: 34; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 54; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 36. In some embodiments, the first scFv domain comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 60; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 62; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 34; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 54; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 36. In certain embodiments, the second scFv domain comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 18; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 4; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 6; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 8; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 12. In some embodiments, the first scFv domain comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 30; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 32; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 34; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 42; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 36. In some embodiments, the second scFv domain comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 24; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 4; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 6; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 8; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 12. In some embodiments, the first scFv domain comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 52; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 32; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 34; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 54; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 36. In some embodiments, the second scFv domain comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 24; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 4; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 6; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 8; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 12, and, optionally, the first scFv domain

comprises: (a) the HCDR1 amino acid sequence set forth in SEQ ID NO: 30; (b) the HCDR2 amino acid sequence set forth in SEQ ID NO: 32; (c) the HCDR3 amino acid sequence set forth in SEQ ID NO: 34; (d) the LCDR1 amino acid sequence set forth in SEQ ID NO: 8; (e) the LCDR2 amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 amino acid sequence set forth in SEQ ID NO: 36.

[0028] The disclosure also provides a multispecific polypeptide wherein a first scFv domain comprises i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 38 and a light chain variable region comprising an amino acid of SEQ ID NO: 40, and wherein a second scFv domain comprises ii) a heavy chain variable region comprising an amino acid of SEQ ID NO: 14 and a light chain variable region of SEQ ID NO: 16. In some embodiments, the first scFv domain comprises i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 38 and a light chain variable region comprising an amino acid of SEQ ID NO: 44, and the second scFv domain comprises ii) a heavy chain variable region comprising an amino acid of SEQ ID NO: 14 and a light chain variable region of SEQ ID NO: 16. In certain embodiments, the first scFv domain comprises i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46 and a light chain variable region comprising an amino acid of SEQ ID NO: 48, and the second scFv domain comprises ii) a heavy chain variable region comprising an amino acid of SEQ ID NO: 14 and a light chain variable region of SEQ ID NO: 16. In other embodiments, the first scFv domain comprises i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 56 and a light chain variable region comprising an amino acid of SEQ ID NO: 58, and the second scFv domain comprises ii) a heavy chain variable region comprising an amino acid of SEQ ID NO: 14 and a light chain variable region of SEQ ID NO: 16. In certain embodiments, the first scFv domain comprises i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 64 and a light chain variable region comprising an amino acid of SEQ ID NO: 58, and the second scFv domain comprises ii) a heavy chain variable region comprising an amino acid of SEQ ID NO: 14 and a light chain variable region of SEQ ID NO: 16. In other embodiments, the first scFv domain comprises i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46 and a light chain variable region comprising an amino acid of SEQ ID NO: 50, and the second scFv domain comprises ii) a heavy chain variable region comprising an amino acid of SEQ ID NO: 20 and a light chain variable region of SEQ ID NO: 22. In yet other embodiments, the first scFv domain comprises i) a heavy chain variable region

comprising an amino acid sequence of SEQ ID NO: 56 and a light chain variable region comprising an amino acid of SEQ ID NO: 58, and the second scFv domain comprises ii) a heavy chain variable region comprising an amino acid of SEQ ID NO: 26 and a light chain variable region of SEQ ID NO: 16. In some embodiments, the first scFv domain comprises i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 38 and a light chain variable region comprising an amino acid of SEQ ID NO: 68, and the second scFv domain comprises ii) a heavy chain variable region comprising an amino acid of SEQ ID NO: 28 and a light chain variable region of SEQ ID NO: 16. In some embodiments, the first scFv domain comprises i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46 and a light chain variable region comprising an amino acid of SEQ ID NO: 70, and the second scFv domain comprises ii) a heavy chain variable region comprising an amino acid of SEQ ID NO: 28 and a light chain variable region of SEQ ID NO: 16.

[0029] In some embodiments, the present disclosure provides a multispecific polypeptide comprising a first scFv domain and a second scFv domain, wherein the first and second scFv domains are linked together by a binding domain linker or a binding domain linker and an immunoglobulin Fc domain, wherein the immunoglobulin Fc domain comprises a hinge region and an immunoglobulin constant region; and wherein the first scFv domain specifically binds to human 5T4 and comprises: (i) an immunoglobulin heavy chain variable region comprising an HCDR1 amino acid sequence of SEQ ID NO: 30, an HCDR2 amino acid sequence of SEQ ID NO: 32, and an HCDR3 amino acid sequence of SEQ ID NO: 34; and (ii) an immunoglobulin light chain variable region comprising an LCDR1 amino acid sequence of SEQ ID NO: 42, an LCDR2 amino acid sequence of SEQ ID NO: 10, and an LCDR3 amino acid sequence of SEQ ID NO: 36.

[0030] In some embodiments, the first scFv domain comprises: i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 38 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 44; or ii) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 48; or iii) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 50.

[0031] In some embodiments, the present disclosure provides a multispecific polypeptide comprising a first scFv domain and a second scFv domain, wherein the first and

second scFv domains are linked together by a binding domain linker or a binding domain linker and an immunoglobulin Fc domain, wherein the immunoglobulin Fc domain comprises a hinge region and an immunoglobulin constant region; and wherein the first scFv domain specifically binds to human 5T4, wherein the VH and/or the VL region of the second scFv domain comprise one or more mutations in the framework region; and wherein the first scFv domain comprises: (a) the HCDR1 comprising an amino acid sequence of SEQ ID NO: 30; (b) the HCDR2 comprising an amino acid sequence of SEQ ID NO: 32; (c) the HCDR3 comprising an amino acid sequence of SEQ ID NO: 34; (d) the LCDR1 comprising an amino acid sequence of SEQ ID NO: 42; (e) the LCDR2 comprising an amino acid sequence of SEQ ID NO: 10; and (f) the LCDR3 comprising an amino acid sequence of SEQ ID NO: 36. In some embodiments, the first scFv domain comprises i) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 38 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 44; or ii) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 48; or iii) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 46 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 50.

[0032] In some embodiments, the multispecific polypeptides described herein comprise, from amino-terminus to carboxyl-terminus, (i) the first scFv domain, (ii) a binding domain linker, and (iii) the second binding domain. In some embodiments, the multispecific polypeptides comprise, from amino-terminus to carboxyl-terminus, (i) the second scFv domain, (ii) a binding domain linker, and (iii) the first scFv domain. In some embodiments, the multispecific polypeptides comprise, from amino-terminus to carboxyl-terminus, (i) the first scFv domain, (ii) a hinge region, (iii) an immunoglobulin constant region, (iv) a binding domain linker, and (v) the second scFv domain. In some embodiments, the binding domain linker comprises a Gly₄Ser sequence. In some embodiments, the binding domain linker comprises the formula (Gly₄Ser)_n, wherein n = 1-5. In some embodiments, the binding domain linker comprises an amino acid sequence selected from SEQ ID NOs: 85-108. In some embodiments, the binding domain linker comprises an amino acid sequence set forth in SEQ ID NO: 107 (SGGGGSGGGGSGGGGSPS).

[0033] In certain aspects, the first scFv domain of a multispecific polypeptide of the disclosure specifically binds to human 5T4 and comprises an amino acid sequence selected

from the group consisting of SEQ ID NOs: 118, 120, 122, 124, 126, 128, 130, 132, 134, and 170; and the second scFv domain specifically binds to human 4-1BB and comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 110, 112, 114, and 116. In some embodiments, the first scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 118 and the second scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 110. In other embodiments, the first scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 120 and the second scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 110. In certain embodiments, the first scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 122 and the second scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 110. In some embodiments, the first scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 124 and the second scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 112. In some embodiments, the first scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 126 and the second scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 114. In other embodiments, the first scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 126 and the second scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 110. In certain embodiments, the first scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 128 and the second scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 110. In some embodiments, the first scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 170 and the second scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 116. In some embodiments, the first scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 130 and the second scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 116. In some embodiments, the first scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 132 and the second scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 116. In other embodiments, the first scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 134 and the second scFv domain comprises an amino acid sequence set forth in SEQ ID NO: 116. In some embodiments, the disclosure provides a multispecific polypeptide, wherein the polypeptide comprises an amino acid sequence comprising at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 172, 174, and 176. In some embodiments,

the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 172, 174, and 176.

[0034] In some embodiments, the disclosure provides a multispecific polypeptide that specifically binds to 5T4 and 4-1BB, wherein the polypeptide comprises an amino acid sequence comprising at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 172, 174, and 176. In certain embodiments, the polypeptide that specifically binds to 5T4 and 4-1BB comprises an amino acid sequence comprising at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 172, 174, and 176, wherein the polypeptide comprises the same CDR amino acid sequences as the respective SEQ ID NO or the polypeptide comprises CDR amino acid sequences that deviate by no more than one amino acid as compared to the respective SEQ ID NO. In some embodiments, the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 172, 174, and 176.

[0035] The disclosure further provides a multispecific polypeptide wherein the first scFv domain binds to an extracellular domain of 5T4 and wherein the second scFv domain binds to an extracellular domain of 4-1BB. In some aspects, the polypeptide results in enhanced effector cell activation. In some aspects, a multispecific polypeptide of the disclosure increases effector cell activation and/or effector cell proliferation. In other aspects, a multispecific polypeptide enhances effector cell-dependent lysis of 5T4-expressing cells. In some embodiments, a 5T4-binding domain of the disclosure is capable of binding 5T4 with a K_D value of less than 50 nM. In some aspects, the light chain variable region and/or the heavy chain variable region of the 5T4-binding domain and/or the 4-1BB-binding domain is humanized.

[0036] In some embodiments, the present disclosure provides a multispecific polypeptide comprising a first single chain variable fragment (scFv) domain and a second scFv domain linked together by a binding domain linker and an immunoglobulin Fc domain, wherein the immunoglobulin Fc domain comprises a hinge region and an immunoglobulin constant region; wherein the multispecific polypeptide is capable of forming a homodimer by association with a second identical multispecific polypeptide; wherein the first scFv domain specifically binds to human 5T4 and comprises: (i) an immunoglobulin heavy chain variable region comprising a heavy

chain complementarity determining region (HCDR)-1 amino acid sequence of SEQ ID NO: 30, an HCDR2 amino acid sequence of SEQ ID NO: 32, and an HCDR3 amino acid sequence of SEQ ID NO: 34; and (ii) an immunoglobulin light chain variable region comprising a light chain complementarity determining region (LCDR)-1 amino acid sequence of SEQ ID NO: 42, an LCDR2 amino acid sequence of SEQ ID NO: 10, and an LCDR3 amino acid sequence of SEQ ID NOs: 36; and wherein the second scFv domain specifically binds to human 4-1BB and comprises: (i) an immunoglobulin heavy chain variable region comprising an HCDR1 amino acid sequence of SEQ ID NO: 2, an HCDR2 amino acid sequence of SEQ ID NO: 4, and an HCDR3 amino acid sequence of SEQ ID NO: 6; and (ii) an immunoglobulin light chain variable region comprising an LCDR1 amino acid sequence of SEQ ID NO: 8, an LCDR2 amino acid sequence of SEQ ID NO: 10, and an LCDR3 amino acid sequence of SEQ ID NO: 12. 2. The multispecific polypeptide of claim 1, wherein the multispecific polypeptide comprises, from amino-terminus to carboxyl-terminus: (i) the first scFv domain, (ii) the hinge region, (iii) the immunoglobulin constant region, (iv) the binding domain linker, and (v) the second scFv domain. In some embodiments, the first scFv domain comprises a mutation in the framework region compared to the framework region of SEQ ID NOs: 130 or 170. In some embodiments, the mutation introduces a stabilizing disulfide bond.

[0037] In some embodiments, the first scFv domain of the multispecific polypeptide comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 38 and the immunoglobulin light chain variable region of SEQ ID NO: 44. In some embodiments, the first scFv domain of the multispecific polypeptide comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 46 and the immunoglobulin light chain variable region of SEQ ID NO: 48. In some embodiments, the second scFv domain of the multispecific polypeptide comprises immunoglobulin heavy chain variable region of SEQ ID NO: 14 and the immunoglobulin light chain variable region of SEQ ID NO: 16. In some embodiments, the first scFv domain of the multispecific polypeptide comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 46 and the immunoglobulin light chain variable region of SEQ ID NO: 48, and the second scFv domain comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 14 and the immunoglobulin light chain variable region of SEQ ID NO: 16. In some embodiments, the amino acid sequence of the first scFv domain is at least 97% identical to SEQ ID NO: 120, and the amino acid sequence of the second scFv domain is at least 97% identical to SEQ ID NO: 110. In

some embodiments, the polypeptide comprises an amino acid sequence at least 95% identical to SEQ ID NO: 172 or comprises an amino acid sequence identical to SEQ ID NO: 172. In some embodiments, the amino acid sequence of the first scFv domain is at least 97% identical to SEQ ID NO: 122 and the amino acid sequence of the second scFv domain is at least 97% identical to SEQ ID NO: 110. In some embodiments, the polypeptide comprises an amino acid sequence at least 95% identical to SEQ ID NO: 174 or comprises an amino acid sequence of SEQ ID NO: 174.

[0038] In some embodiments, binding of the multispecific polypeptide to an effector cell results in increased effector cell activation, increased effector cell proliferation, or wherein binding of the multispecific polypeptide to an effector cell an a 5T4-expressing cell results in enhanced effector cell-dependent lysis of the 5T4-expressing cell.

[0039] The disclosure also encompasses a dimer comprising two identical polypeptides, wherein the two polypeptides are each the multispecific polypeptide described in the disclosure.

[0040] In certain aspects, the disclosure provides a pharmaceutical composition comprising a polypeptide or a protein described in the disclosure, and a pharmaceutically acceptable carrier, diluent, or excipient. In some embodiments, a pharmaceutical composition may be formulated in a dosage form selected from the group consisting of: an oral unit dosage form, an intravenous unit dosage form, an intranasal unit dosage form, a suppository unit dosage form, an intradermal unit dosage form, an intramuscular unit dosage form, an intraperitoneal unit dosage form, a subcutaneous unit dosage form, an epidural unit dosage form, a sublingual unit dosage form, and an intracerebral unit dosage form. Non-limiting examples of an oral unit dosage form include tablets, pills, pellets, capsules, powders, lozenges, granules, solutions, suspensions, emulsions, syrups, elixirs, sustained-release formulations, aerosols, and sprays.

[0041] The disclosure further encompasses a method for enhancing effector cell activation against a cell expressing 5T4, the method comprising: contacting said 5T4-expressing cell with a polypeptide or a protein of the disclosure, wherein said contacting is under conditions whereby enhanced effector cell activation against the 5T4-expressing cell is induced. In some aspects, the disclosure provides a method for treating a disorder in a subject, wherein said disorder is characterized by expression of 5T4, the method comprising administering to the subject a therapeutically effective amount of a polypeptide or a protein or a pharmaceutical composition of the disclosure. The disclosure also encompasses a use of a polypeptide or a protein of the disclosure

for the manufacture of a medicament for treatment of a disorder in a subject, wherein said disorder is characterized by expression of 5T4. In some embodiments, the disclosure is related to a polypeptide or a protein of the disclosure for use in treating a disorder in a subject, wherein said disorder is characterized by expression of 5T4. In some embodiments, the disorder is a cancer. In certain aspects, the cancer is breast cancer, pancreatic cancer, ovarian cancer, non-small cell lung cancer, mesothelioma, chronic lymphocytic leukemia (CLL), mantle cell leukemia (MCL), acute lymphoblastic leukemia (ALL), squamous cell carcinoma, melanoma, adrenal cancer, bladder cancer, cervical cancer, renal cancer, gastric cancer, prostate cancer, thyroid cancer, liver cancer, uterine cancer, neurofibroma, sarcoma or head and neck cancer.

[0042] In some embodiments, the multispecific polypeptide for use in such methods comprises, from amino-terminus to carboxyl-terminus: (i) the first scFv domain, (ii) the hinge region, (iii) the immunoglobulin constant region, (iv) the binding domain linker, and (v) the second scFv domain. In some embodiments, the multispecific polypeptide for use in such methods comprises a second scFv domain comprising an immunoglobulin heavy chain variable region of SEQ ID NO: 14 and an immunoglobulin light chain variable region of SEQ ID NO: 16, and a first scFv domain comprising (i) the immunoglobulin heavy chain variable region of SEQ ID NO: 38 and the immunoglobulin light chain variable region of SEQ ID NO: 44; or (ii) the immunoglobulin heavy chain variable region of SEQ ID NO: 46 and the immunoglobulin light chain variable region of SEQ ID NO: 48. In some embodiments, the amino acid sequence of the second scFv domain of the multispecific polypeptide is at least 97% identical to SEQ ID NO: 110, and the amino acid sequence of the first scFv domain of the multispecific polypeptide is at least 97% identical to SEQ ID NO: 120 (anti-5T4 scFv for 209) or SEQ ID NO: 122. In some embodiments, said polypeptide exhibits statistically significant enhanced effector cell activation compared to a second multispecific polypeptide, wherein the second multispecific polypeptide is an IgG-scFv structure comprising an anti-4-1BB antibody comprising a variable heavy chain comprising SEQ ID NO: 28 and a variable light chain comprising SEQ ID NO: 16 and an anti-5T4 scFv comprising a variable heavy chain comprising SEQ ID NO: 46 and a variable light chain comprising SEQ ID NO: 66. In some embodiments, said polypeptide induces statistically significant increased effector cell proliferation compared to a second multispecific polypeptide, wherein the second multispecific polypeptide is an IgG-scFv structure comprising an anti-4-1BB antibody comprising a variable heavy chain comprising SEQ ID NO: 28 and a variable light chain comprising SEQ ID

NO: 16 and an anti-5T4 scFv comprising a variable heavy chain comprising SEQ ID NO: 46 and a variable light chain comprising SEQ ID NO: 66.

[0043] Some aspects of the disclosure include an isolated nucleic acid molecule encoding a polypeptide of the disclosure. In some embodiments, a nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 171, 173, and 175. The disclosure encompasses an expression vector comprising an isolated nucleic acid molecule described herein. In some embodiments, the nucleic acid molecule in an expression vector is operatively linked to regulatory sequences suitable for expression of the nucleic acid segment in a host cell. The disclosure provides a recombinant host cell comprising an expression vector described herein. In some aspects, the disclosure provides a method for producing a polypeptide comprising a 5T4-binding domain, the method comprising culturing a recombinant host cell comprising an expression vector described herein under conditions whereby the nucleic acid segment is expressed, thereby producing the polypeptide comprising a 5T4-binding domain.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] FIG. 1 illustrates the agonistic function of seven constructs (ALG.APV-004, ALG.APV-006, ALG.APV-099, ALG.APV-127, ALG.APV-148, and ALG.APV-150) in the presence of 5T4(+) cells.

[0045] FIG. 2 illustrates binding curves of seven scFv-Fc-scFv molecules (ALG.APV-006, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-191, ALG.APV-196, and ALG.APV-198) to the CHO-K1 cell line expressing human 4-1BB.

[0046] FIG. 3 illustrates binding curves of seven scFv-Fc-scFv molecules (ALG.APV-006, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-191, ALG.APV-196 and ALG.APV-198) to the CHO-K1 cell line expressing cynomolgus 4-1BB.

[0047] FIG. 4 illustrates the binding curves of seven scFv-Fc-scFv molecules (ALG.APV-006, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-191, ALG.APV-196, and ALG.APV-198) to the CHO-K1 cell line expressing human 5T4.

[0048] FIG. 5 illustrates the expression levels of human 5T4 in the CHO-K1 and SKOV-3 cell lines.

[0049] FIG. 6 illustrates the binding curves of the ALG.APV-006, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-196, and ALG.APV-198 constructs on SKOV-3 cells.

[0050] FIG. 7 illustrates the binding curves of ALG.APV-006, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-191, ALG.APV-196, and ALG.APV-198 on the CHO-K1 cell line expressing cynomolgus 5T4.

[0051] FIG. 8 illustrates the activity of anti-5T4 x anti-4-1BB constructs in a 4-1BB reporter assay.

[0052] FIG. 9 illustrates the effect of anti-5T4 x anti-4-1BB constructs on IFN γ release from PBMC and CHO-K1 co-cultures.

[0053] FIG. 10 illustrates an exemplary embodiment of a protein with an scFv-Fc-scFv format (ADAPTIRTM format).

[0054] FIG. 11A – FIG. 11B show the interferon gamma (IFN γ) response in human CD8+ T cells cultured with bispecific scFv-Fc-scFv constructs. FIG. 11A shows T cell responses when cultured in plates coated with 5T4-Fc antigen. FIG. 11B shows T cell responses when cultured without 5T4-Fc antigen. The figure displays mean values from two donors.

[0055] FIG. 12 shows the binding curves of ALG.APV-004, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-208, ALG.APV-209, and ALG.APV-210 to the CHO-K1/human CD137 cell line.

[0056] FIG. 13 shows the binding curves of ALG.APV-004, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-208, ALG.APV-209, and ALG.APV-210 to murine CT26 cells (ATCC) expressing human 5T4.

[0057] FIG. 14 shows the binding curves of ALG.APV-004, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-208, ALG.APV-209 and ALG.APV-210 on the CHO-K1/cynomolgus 5T4 transfectants.

[0058] FIG. 15 shows the binding curves of ALG.APV-004, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-208, ALG.APV-209 and ALG.APV-210 on a human cell line expressing neither CD137 nor 5T4 (MOLM13 cells, ATCC).

[0059] FIG. 16 shows the activity of the bispecific constructs in Jurkat/NF- κ B reporter cells, after 5 hours of incubation in the presence of 5T4(+) cells (HCC1143) or 5T4(-) cells (MOLM13). Every point in the curve represents the average of duplicate wells. The y-axis shows values in relative fluorescence units (RLU).

[0060] FIG. 17 shows the levels of IFN- γ induced in primary PBMC cultures at 72 by ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-208, ALG.APV-209, ALG.APV-210, and the Morrison format control construct, ALG.APV-004. Every point in the curve represents the average of duplicate wells.

[0061] FIG. 18A – FIG. 18B illustrates FACS analysis of 5T4-dependent localization of bispecific constructs ALG.APV-209, ALG.APV-210, and ALG.APV-004 to antigen-expressing tumors. Localization was detected by staining with an anti-human Fc antibody (FIG. 18A) or biotinylated 4-1BB (FIG. 18B).

[0062] FIG. 19A – FIG. 19B illustrates immunohistochemical analysis of 5T4-dependent localization of bispecific constructs ALG.APV-209, ALG.APV-210, and ALG.APV-004 to 5T4+ (FIG. 19A) and 5T4- (FIG. 19B) tumors.

[0063] FIG. 20 shows the IFN γ response of human CD8 T cells cultured with the bispecific antibody ALG.APV-210 in the presence of CT26 cells expressing different levels of 5T4.

[0064] FIG. 21A – FIG. 21B show the maximum IFN γ release of human CD8 T cells cultured with the bispecific antibody ALG.APV-210 in the presence of CT26 cells expressing different levels of 5T4.

[0065] FIG. 22A – FIG. 22C show ALG.APV-210 or ANC107 (isotype control) binding to primary CD8 T cells gated from CD3-stimulated or unstimulated human and cynomolgus PBMC. The MFI (and SEM) from 2 independent experiments are shown in FIG. 22A and FIG. 22B. FIG. 22C shows the normalized pooled data for ALG.APV-210 from the 2 independent experiments. n=3 donors/group/exp.

[0066] FIG. 23A – FIG. 23C show ALG.APV-210 or ANC107 (isotype control) binding to primary CD8 T cells gated from CD3-stimulated or unstimulated human and cynomolgus PBMC. Percentage and SEM of ALG.APV-210 binding to CD8 T cells from 2 independent experiments are shown in FIG. 23A and 23B. FIG. 23C shows the normalized pooled data from experiment 1 and 2. n= 3 donors/group/exp.

[0067] FIG. 24 shows the agonistic function of ALG.APV-210 on human CD8 T cells.

[0068] FIG. 25A – FIG. 25B show the agonistic function of ALG.APV-210 on human CD8 T cells from individual representative donors.

[0069] FIG. 26 shows the agonistic function of ALG.APV-210 on cynomolgus CD8 T cells.

[0070] FIG. 27A – FIG. 27C show the agonistic function of ALG.APV-210 on cynomolgus CD8 T cells from individual representative donors. The figures show a dose response dependent IFN γ production by cynomolgus CD8 T activated with ALG.APV-210 in the presence of 5T4-Fc.

[0071] FIG. 28 shows the anti-tumor efficacy of a bispecific antibody ALG.APV-210 in HCT-116, a 5T4 positive human colon carcinoma xenograft tumor model in SCID beige mice.

[0072] FIG. 29 shows the PK analysis of ALG.APV-210 or ALG.APV-209.

[0073] FIG. 30A – FIG. 30B show the binding of the Fc portion of ALG.APV-210 to Fc γ R. Titrated ALG.APV-210 was incubated with the Fc γ R-expressing cells and probed with a fluorescently-labelled anti-human IgG secondary antibody (FIG. 30A). Human IgG1 molecule was used as a positive control (FIG. 30B).

[0074] FIG. 31A – FIG. 31B show the CD8+ and CD4+ T-cell proliferation induced by the bispecific molecules ALG.APV-209 and ALG.APV-210 in the presence of CHO-K1 cells expressing human 5T4 and anti-CD3 antibodies.

[0075] FIG. 32A – FIG. 32B show induction of IFN- γ secretion by the bispecific molecule ALG.APV-210 in whole PBMC cultures, from two different human PBMC donors (A and B).

[0076] FIG. 33A – FIG. 33B show CD8+ T-cell proliferation induced by the bispecific molecule ALG.APV-210 in whole PBMC cultures, from two different human PBMC donors.

[0077] FIG. 34 shows binding of ALG.APV-210 to 5T4-expressing cell lines.

[0078] FIG. 35 shows agonist function of the bispecific construct ALG.APV-210 in Jurkat/NF- κ B reporter cells after incubation in the presence of cells expressing a range of surface 5T4 protein densities.

[0079] FIG. 36 shows the IFN γ response of human CD8+ T cells cultured with the bispecific antibody ALG.APV-210 in the presence of human HCT116 cells expressing 5T4.

[0080] FIG. 37 shows IFN γ response of human CD8+ T cells cultured with the bispecific antibody ALG.APV-210 in the presence of human HCT116 cells expressing 5T4.

[0081] FIG. 38 shows the maximum IFN γ release of human CD8 T cells cultured with the bispecific antibody ALG.APV-210 in the presence of HCT116 cells expressing human 5T4.

[0082] FIG. 39A – FIG. 39D show ALG.APV-210 binding to 5T4 expressing human tumor cells or transfected cell lines.

[0083] FIG. 40A – FIG. 40D show normalized MFI for experiments demonstrating ALG.APV-210 binding to 5T4 expressing human tumor cells or transfected cell lines.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0084] The disclosure provides polypeptides comprising binding domains that specifically bind to trophoblast glycoprotein (5T4) and polypeptides that specifically bind to 5T4. In some embodiments, the polypeptides are multispecific polypeptides that may bind specifically to 5T4 and to another target. In some embodiments, the polypeptides described herein are multispecific polypeptides that bind specifically to 5T4 and also bind specifically to a target on an effector cell. The disclosure also provides polypeptides comprising binding domains that specifically bind to tumor necrosis factor receptor superfamily member 9 (41BB or CD137) and polypeptides that specifically bind to 4-1BB. In some embodiments, the polypeptides are multispecific polypeptides that may bind specifically to 4-1BB and to another target (e.g., a tumor-associated antigen). In some embodiments, the polypeptides described herein are multispecific polypeptides that bind specifically to 4-1BB and also bind specifically to a target on a target cell. In some embodiments, the multispecific polypeptides are bispecific polypeptides that bind specifically to 5T4 and bind specifically to 4-1BB. In some embodiments, the bispecific polypeptides bind to 5T4 expressed on a target cell and 4-1BB expressed on an effector cell, thereby resulting in amplification of effector cell activation and enhancing effector cell-mediated cytotoxicity of a target cell.

[0085] In some embodiments, the present disclosure provides 5T4-binding domains and/or 4-1BB-binding domains (and polypeptides or proteins comprising such binding domains) that exhibit less off-target binding relative to previously known 5T4-binding domains and/or 4-1BB-binding domains. In certain aspects, the binding domains and/or polypeptides comprising the binding domains described herein bind to 5T4 and/or 4-1BB more effectively in

certain formats and/or certain orientations (*e.g.*, V_H-V_L compared to V_L-V_H), leading to higher potency and/or improved utility in treating disorders associated with expression of 5T4.

[0086] In some embodiments, administration of a therapeutically effective amount of a polypeptide or protein described herein to a patient in need thereof is useful for treatment of certain disorders associated with the expression of 5T4, including certain cancers. In one embodiment, the polypeptide or protein binds both a target cell expressing 5T4 and an effector-cell, thereby “cross-linking” the target cell expressing 5T4 and the effector cell. The binding of both domains to their targets enhances the activation of the effector cells, leading to a prolonged and/or more robust effector cell response (*e.g.*, effector cell-mediated cytotoxicity). The polypeptides and proteins of the present disclosure offer various advantages in treating patients, for example, effective binding to 5T4, efficient enhancement of effector cell activity, reduced levels of cytokine release, and/or a lower risk of adverse events (*e.g.*, toxicity). In some embodiments, a target cell expresses 5T4 at a higher level than a non-target cell (*e.g.*, normal cell or non-cancerous cell in the same subject, organ, or tissue) expresses 5T4. In other embodiments, a target cell expresses 5T4 while a non-target cell (*e.g.*, normal cell or non-cancerous cell in the same subject, organ, or tissue) does not express 5T4.

[0087] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited herein, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. In the event that one or more of the incorporated documents or portions of documents define a term that contradicts that term’s definition in the application, the definition that appears in this application controls. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment, or any form of suggestion, that they constitute valid prior art or form part of the common general knowledge in any country in the world.

[0088] In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. It should be understood that the terms “a” and “an” as used herein refer to “one or more” of the enumerated components unless otherwise indicated. The use of the

alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include" and "comprise" are used synonymously. In addition, it should be understood that the polypeptides comprising the various combinations of the components (*e.g.*, domains or regions) and substituents described herein, are disclosed by the present application to the same extent as if each polypeptide was set forth individually. Thus, selection of particular components of individual polypeptides is within the scope of the present disclosure.

Definitions

[0089] As used herein, the term "binding domain" or "binding region" refers to the domain, region, portion, or site of a protein, polypeptide, oligopeptide, peptide, antibody, or binding domain derived from an antibody that possesses the ability to specifically recognize and bind to a target molecule, such as an antigen, ligand, receptor, substrate, or inhibitor (*e.g.*, 5T4 or 4-1BB). Exemplary binding domains include, antibodies and antibody-like proteins or domains, antibody heavy and light chain variable regions, and single-chain antibody variable regions (*e.g.*, domain antibodies, sFv, scFv, scFab). In certain embodiments, the binding domain comprises or consists of an antigen binding site (*e.g.*, comprising a variable heavy chain sequence and variable light chain sequence or three light chain complementary determining regions (CDRs) and three heavy chain CDRs from an antibody placed into alternative framework regions (FRs) (*e.g.*, human FRs optionally comprising one or more amino acid substitutions). A variety of assays are known for identifying binding domains of the present disclosure that specifically bind a particular target, including Western blot, ELISA, phage display library screening, and BIACORE® interaction analysis. In some embodiments, the polypeptides of the present invention comprise a binding domain that specifically binds to a target antigen expressed by a target cell (*e.g.*, a tumor associated antigen, such as 5T4). In some embodiments, the polypeptides of the present invention comprise a binding domain that specifically binds to a target antigen expressed by an effector cell (*e.g.*, 4-1BB). In some embodiments, the polypeptides of the present invention are multispecific polypeptides and comprise two or more binding domains.

[0090] A binding domain or protein comprising a binding domain "specifically binds" a target if it binds the target with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of $1/M$) equal to or greater than $10^5 M^{-1}$, while not

significantly binding other components present in a test sample. Binding domains can be classified as “high affinity” binding domains and “low affinity” binding domains. “High affinity” binding domains refer to those binding domains with a K_a of at least 10^7 M^{-1} , at least 10^8 M^{-1} , at least 10^9 M^{-1} , at least 10^{10} M^{-1} , at least 10^{11} M^{-1} , at least 10^{12} M^{-1} , or at least 10^{13} M^{-1} . “Low affinity” binding domains refer to those binding domains with a K_a of up to 10^7 M^{-1} , up to 10^6 M^{-1} , up to 10^5 M^{-1} . Alternatively, affinity can be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (e.g., 10^{-5} M to 10^{-13} , or about 500 nM, about 300 nM, about 250 nM, about 200 nM, about 150 nM, about 100 nM, about 50 nM, about 25 nM, about 10 nM, or about 5 nM). Affinities of binding domain polypeptides and single chain polypeptides according to the present disclosure can be readily determined using conventional techniques (*see, e.g.*, Scatchard et al. (1949) Ann. N.Y. Acad. Sci. 51:660; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

[0091] As used herein, a “conservative substitution” is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are well-known in the art (*see, e.g.*, PCT Application Publication No. WO 97/09433, page 10, published March 13, 1997; Lehninger, Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77; Lewin, Genes IV, Oxford University Press, NY and Cell Press, Cambridge, MA (1990), p. 8).

[0092] As used herein, the term “derivative” refers to a modification of one or more amino acid residues of a peptide by chemical or biological means, either with or without an enzyme, e.g., by glycosylation, alkylation, acylation, ester formation, or amide formation.

[0093] As used herein, a polypeptide or amino acid sequence “derived from” a designated polypeptide or protein refers to the origin of the polypeptide. In certain embodiments, the polypeptide or amino acid sequence which is derived from a particular sequence (sometimes referred to as the “parent” or “parental” sequence) and has an amino acid sequence that is essentially identical to the parent sequence or a portion thereof, wherein the portion consists of at least 10-20 amino acids, at least 20-30 amino acids, or at least 30-50 amino acids, or at least 50-150 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the parent sequence. For example, a binding domain (e.g., a Fab, F(ab')₂, Fab', scFv, single domain antibody (sdAb), etc.) can be derived from an antibody. In some embodiments, a

binding domain sequence (*e.g.*, a 5T4- or 4-1BB-binding domain) is derived from an antibody or protein by means of a computer algorithm or *in silico*.

[0094] Polypeptides derived from another polypeptide can have one or more mutations or alterations relative to the parent polypeptide, *e.g.*, one or more amino acid residues which have been substituted with another amino acid residue or which has one or more amino acid insertions or deletions. In such embodiments, polypeptides derived from a parent polypeptide and comprising one or more mutations or alteration are referred to as “variants.” As used herein, the term “variant” or “variants” refers to a polynucleotide or polypeptide with a sequence differing from that of a reference polynucleotide or polypeptide, but retaining essential properties thereof. Generally, variant polynucleotide or polypeptide sequences are overall closely similar, and, in many regions, identical to the reference polynucleotide or polypeptide. For instance, a variant polynucleotide or polypeptide may exhibit at least about 70%, at least about 80%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% sequence identity compared to the active portion or full length reference polynucleotide or polypeptide. The polypeptide can comprise an amino acid sequence which is not naturally occurring. Such variations necessarily have less than 100% sequence identity or similarity with the parent polypeptide. In one embodiment, the variant will have an amino acid sequence from about 60% to less than 100% amino acid sequence identity or similarity with the amino acid sequence of the parent polypeptide. In another embodiment, the variant will have an amino acid sequence from about 75% to less than 100%, from about 80% to less than 100%, from about 85% to less than 100%, from about 90% to less than 100%, from about 95% to less than 100% amino acid sequence identity or similarity with the amino acid sequence of the parent polypeptide.

[0095] As used herein, the term “sequence identity” refers to a relationship between two or more polynucleotide sequences or between two or more polypeptide sequences. When a position in one sequence is occupied by the same nucleic acid base or amino acid residue in the corresponding position of the comparator sequence, the sequences are said to be “identical” at that position. The percentage sequence identity is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of identical positions. The number of identical positions is then divided by the total number of positions in the comparison window and multiplied by 100 to yield the percentage of sequence

identity. Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window. The comparison window for polynucleotide sequences can be, for instance, at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 or more nucleic acids in length. The comparison window for polypeptide sequences can be, for instance, at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300 or more amino acids in length. In order to optimally align sequences for comparison, the portion of a polynucleotide or polypeptide sequence in the comparison window can comprise additions or deletions termed gaps while the reference sequence is kept constant. An optimal alignment is that alignment which, even with gaps, produces the greatest possible number of “identical” positions between the reference and comparator sequences. Percentage “sequence identity” between two sequences can be determined using the version of the program “BLAST 2 Sequences” which was available from the National Center for Biotechnology Information as of September 1, 2004, which program incorporates the programs BLASTN (for nucleotide sequence comparison) and BLASTP (for polypeptide sequence comparison), which programs are based on the algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90(12):5873-5877, 1993). When utilizing “BLAST 2 Sequences,” parameters that were default parameters as of September 1, 2004, can be used for word size (3), open gap penalty (11), extension gap penalty (1), gap dropoff (50), expect value (10) and any other required parameter including but not limited to matrix option. Two nucleotide or amino acid sequences are considered to have “substantially similar sequence identity” or “substantial sequence identity” if the two sequences have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity relative to each other.

[0096] As used herein, unless otherwise provided, a position of an amino acid residue in a variable region of an immunoglobulin molecule is numbered according to either the IMGT criteria (Brochet *et al.*, Nucl. Acids Res. (2008) 36, W503-508) or according to EU nomenclature (Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94), and a position of an amino acid residue in a constant region of an immunoglobulin molecule is numbered according to EU nomenclature (Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94). The Kabat numbering convention (Kabat, Sequences of Proteins of Immunological Interest, 5th ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)) is an alternative system used to refer to a position of

an amino acid residue in a variable region of an immunoglobulin molecule and is sometimes used to refer to a position of an amino acid residue in a variable region of an immunoglobulin molecule herein.

[0097] As used herein, the term “dimer” refers to a biological entity that consists of two subunits associated with each other via one or more forms of intramolecular forces, including covalent bonds (*e.g.*, disulfide bonds) and other interactions (*e.g.*, electrostatic interactions, salt bridges, hydrogen bonding, and hydrophobic interactions), and is stable under appropriate conditions (*e.g.*, under physiological conditions, in an aqueous solution suitable for expressing, purifying, and/or storing recombinant proteins, or under conditions for non-denaturing and/or non-reducing electrophoresis). The terms “heterodimer” or “heterodimeric protein,” as used herein, refers to a dimer formed from two different polypeptides. A heterodimer may comprise an anti-5T4 x anti-4-1BB molecule as described herein. A heterodimer does not include an antibody formed from four polypeptides (*i.e.*, two light chains and two heavy chains). The terms “homodimer” or “homodimeric protein,” as used herein, refers to a dimer formed from two identical polypeptides.

[0098] “Fc region” or “Fc domain” refers to a polypeptide sequence corresponding to or derived from the portion of a source antibody that is capable of binding to Fc receptors on cells and/or the C1q component of complement, thereby mediating the effector function of an antibody. Fc stands for “fragment crystalline,” the fragment of an antibody that will readily form a protein crystal. Distinct protein fragments, which were originally described by proteolytic digestion, can define the overall general structure of an immunoglobulin protein. As originally defined in the literature, the Fc region is a homodimeric protein comprising two polypeptides that are associated by disulfide bonds, and each comprising a hinge region, a CH2 domain, and a CH3 domain. However, more recently the term has been applied to the single chain monomer component consisting of CH3, CH2, and at least a portion of the hinge sufficient to form a disulfide-linked dimer with a second such chain. As such, and depending on the context, use of the terms “Fc region” or “Fc domain” will refer herein to either the dimeric form or the individual monomers that associate to form the dimeric protein. For a review of immunoglobulin structure and function, see Putnam, *The Plasma Proteins*, Vol. V (Academic Press, Inc., 1987), pp. 49-140; and Padlan, *Mol. Immunol.* 31:169-217, 1994. As used herein, the term Fc includes variants of naturally occurring sequences.

[0099] An “immunoglobulin constant region” or “constant region” is a term defined herein to refer to a peptide or polypeptide sequence that corresponds to or is derived from part or all of one or more constant domains of an immunoglobulin. In certain embodiments, the constant region comprises IgG CH2 and CH3 domains, *e.g.*, IgG1 CH2 and CH3 domains. In certain embodiments, the constant region does not comprise a CH1 domain. In certain embodiments, the constant domains making up the constant region are human. In some embodiments (for example, in certain variations of a 41BB-binding polypeptide, 5T4-binding polypeptide or multispecific polypeptides thereof), the constant region of a fusion protein of this disclosure lacks or has minimal effector functions while retaining the ability to bind some Fc receptors such as the neonatal Fc receptor (FcRn) and retaining a relatively long half-life *in vivo*. For example, the constant region of a fusion protein of this disclosure do not result in, or substantially reduce the induction of antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), complement activation, and/or complement-dependent cytotoxicity (CDC). In other variations, a fusion protein of this disclosure comprises constant domains that retain one or more effector functions, such as of one or both of ADCC and CDC. In certain embodiments, a binding domain of this disclosure is fused to a human IgG1 constant region, wherein the IgG1 constant region has one or more of the following amino acids mutated: leucine at position 234 (L234), leucine at position 235 (L235), glycine at position 237 (G237), glutamate at position 318 (E318), lysine at position 320 (K320), lysine at position 322 (K322), or any combination thereof (numbering according to EU). For example, any one or more of these amino acids can be changed to alanine. In a further embodiment, an IgG1 Fc domain has each of L234, L235, G237, E318, K320, and K322 (according to EU numbering) mutated to an alanine (*i.e.*, L234A, L235A, G237A, E318A, K320A, and K322A, respectively), and optionally an N297A mutation as well (*i.e.*, essentially eliminating glycosylation of the CH2 domain).

[00100] The terms “light chain variable region” (also referred to as “light chain variable domain” or “V_L”) and “heavy chain variable region” (also referred to as “heavy chain variable domain” or “V_H”) refer to the variable binding region from an antibody light and heavy chain, respectively. The variable binding regions are made up of discrete, well-defined sub-regions known as “complementarity determining regions” (CDRs) and “framework regions” (FRs). In one embodiment, the FRs are humanized. The term “CL” refers to an “immunoglobulin light chain constant region” or a “light chain constant region,” *i.e.*, a constant region from an antibody light

chain. The term “CH” refers to an “immunoglobulin heavy chain constant region” or a “heavy chain constant region,” which is further divisible, depending on the antibody isotype into CH1, CH2, and CH3 (IgA, IgD, IgG), or CH1, CH2, CH3, and CH4 domains (IgE, IgM). A "Fab" (fragment antigen binding) is the part of an antibody that binds to antigens and includes the variable region and CH1 domain of the heavy chain linked to the light chain via an inter-chain disulfide bond.

[00101] As used herein, the term “linker” generally refers to a short polypeptide sequence connecting two sub-domains of a polypeptide. Non-limiting examples of linkers include flexible linkers comprising glycine-serine repeats, and linkers derived from (a) an interdomain region of a transmembrane protein (*e.g.*, a type I transmembrane protein); (b) a stalk region of a type II C-lectin; or (c) an immunoglobulin hinge. In some embodiments, a linker provides a spacer function compatible with interaction of the two sub-binding domains so that the resulting polypeptide retains a specific binding affinity to the same target molecule as an antibody that comprises the same light and heavy chain variable regions. In certain embodiments, a linker is comprised of five to about 35 amino acids, for instance, about 15 to about 25 amino acids. As used herein, the phrase a “linker between CH3 and CH1 or CL” refers to one or more amino acid residues (*e.g.*, about 2-12, about 2-10, about 4-10, about 5-10, about 6-10, about 7-10, about 8-10, about 9-10, about 8-12, about 9-12, or about 10-12) between the C-terminus of a CH3 domain (*e.g.*, a wild type CH3 or a mutated CH3) and the N-terminus of a CH1 domain or CL domain (*e.g.*, C κ).

[00102] In some embodiments, depending on context, a linker may refer to (1) a polypeptide region between V_H and V_L regions in a single-chain Fv (scFv) or (2) a polypeptide region between a first binding domain and a second binding domain in a multispecific polypeptide comprising two binding domains. In the later example, wherein a linker connects two or more binding domains, such a linker is referred to herein as a “binding domain linker.” In some embodiments, a binding domain linker may directly link or connect two or more binding domains, resulting in a construct comprising the following structure: binding domain – binding domain linker – binding domain. In some embodiments, the multispecific polypeptides described herein comprise, in order from amino-terminus to carboxyl-terminus (i) a first binding domain, (ii) a binding domain linker, and (iii) a second binding domain. In some embodiments, a multispecific polypeptide comprises, in order from amino-terminus to carboxyl-terminus (i) a second binding

domain, (ii) a binding domain linker, and (iii) a first binding domain. In some embodiments, a binding domain linker may link or connect two or more binding domains by linking at least one binding domain to a non-binding domain polypeptide, such as an immunoglobulin Fc domain (*i.e.*, a polypeptide comprising the structure: Ig hinge – Ig constant region). In such embodiments, the resulting constructs may comprise the following structure: binding domain – Fc domain – binding domain linker – binding domain. In some embodiments, the multispecific polypeptides described herein comprise, in order from amino-terminus to carboxyl-terminus: (i) a first binding domain, (ii) a hinge region, (iii) an immunoglobulin constant region, (iv) a binding domain linker, and (v) a second binding domain. In some embodiments, a multispecific polypeptide comprises, in order from amino-terminus to carboxyl-terminus (i) a second binding domain, (ii) a binding domain linker, (iii) an immunoglobulin constant region, (iv) a hinge region, and (v) a first binding domain. A polypeptide region between an immunoglobulin constant region and a second binding domain in a multispecific polypeptide comprising two binding domains (*e.g.*, a binding domain linker) may also be referred to as a “carboxyl-terminus linker” or an “amino-terminus linker” depending on the orientation of the domains within the multispecific polypeptide. Non-limiting examples of linkers are provided in Table 1.

[00103] In some embodiments, a “hinge” or a “hinge region” refers to a polypeptide derived from an immunoglobulin hinge region and located between a binding domain (*e.g.*, a 5T4-binding domain or a 4-1BB-binding domain) and an immunoglobulin constant region in a polypeptide described herein. A “wild-type immunoglobulin hinge region” refers to a naturally occurring upper and middle hinge amino acid sequences interposed between and connecting the CH1 and CH2 domains (for IgG, IgA, and IgD) or interposed between and connecting the CH1 and CH3 domains (for IgE and IgM) found in the heavy chain of an antibody. In certain embodiments, a wild type immunoglobulin hinge region sequence is human, and can comprise a human IgG hinge region (*e.g.*, and IgG1, IgG2, IgG3, or IgG4 hinge region).

[00104] An “altered immunoglobulin hinge region” or “variant immunoglobulin hinge region” refers to a hinge region polypeptide with one or more mutations, substitutions, insertions, or deletions compared to a corresponding parental wild-type immunoglobulin hinge region. In certain embodiments, an altered immunoglobulin hinge region is at least 70% homologous to a wild-type immunoglobulin hinge region (*e.g.*, at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% homologous). In certain embodiments, an altered immunoglobulin

hinge region is a fragment of a wild type immunoglobulin hinge region that has a length of about 5 amino acids (*e.g.*, about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids) up to about 120 amino acids (for instance, having a length of about 10 to about 40 amino acids or about 15 to about 30 amino acids or about 15 to about 20 amino acids or about 20 to about 25 amino acids). Typically, an altered immunoglobulin hinge region that is a fragment of a wild type immunoglobulin hinge region comprises an IgG core hinge region (*e.g.*, a polypeptide comprising the sequence C-X-X-C, wherein X is any amino acid) as disclosed in U.S. Patent Application Publication Nos. 2013/0129723 and 2013/0095097. Non-limiting examples of hinges are provided in Table 1.

[00105] As used herein, the term “humanized” refers to a process of making an antibody or immunoglobulin binding proteins and polypeptides derived from a non-human species (*e.g.*, mouse or rat) less immunogenic to humans, while still retaining antigen-binding properties of the original antibody, using genetic engineering techniques. In some embodiments, the binding domain(s) of an antibody or immunoglobulin binding proteins and polypeptides (*e.g.*, light and heavy chain variable regions, Fab, scFv) are humanized. Non-human binding domains can be humanized using techniques known as CDR grafting (Jones *et al.*, Nature 321:522 (1986)) and variants thereof, including “reshaping” (Verhoeyen, *et al.*, 1988 Science 239:1534-1536; Riechmann, *et al.*, 1988 Nature 332:323-337; Tempest, *et al.*, Bio/Technol 1991 9:266-271), “hyperchimerization” (Queen, *et al.*, 1989 Proc Natl Acad Sci USA 86:10029-10033; Co, *et al.*, 1991 Proc Natl Acad Sci USA 88:2869-2873; Co, *et al.*, 1992 J Immunol 148:1149-1154), and “veneering” (Mark, *et al.*, “Derivation of therapeutically active humanized and veneered anti-CD18 antibodies.” In: Metcalf BW, Dalton BJ, eds. Cellular adhesion: molecular definition to therapeutic potential. New York: Plenum Press, 1994: 291-312). If derived from a non-human source, other regions of the antibody or immunoglobulin binding proteins and polypeptides, such as the hinge region and constant region domains, can also be humanized. Knowledge about humanized antibodies in the art is applicable to the polypeptides according to the disclosure, even if these polypeptides are not antibodies.

[00106] As used herein, the term “patient in need” refers to a patient at risk of, or suffering from, a disease, disorder or condition that is amenable to treatment or amelioration with a 5T4-binding protein or multispecific polypeptide or a composition thereof provided herein.

[00107] As used herein, the term "pharmaceutically acceptable" refers to molecular entities and compositions that do not generally produce allergic or other serious adverse reactions when administered using routes well known in the art. Molecular entities and compositions approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans are considered to be "pharmaceutically acceptable."

[00108] As used herein, the term "promoter" refers to a region of DNA involved in binding RNA polymerase to initiate transcription.

[00109] As used herein, the terms "nucleic acid," "nucleic acid molecule," or "polynucleotide" refer to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the terms encompass nucleic acids containing analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions) and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions can be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.* (1991) *Nucleic Acid Res.* 19:5081; Ohtsuka *et al.* (1985) *J. Biol. Chem.* 260:2605-2608; Cassol *et al.* (1992); Rossolini *et al.* (1994) *Mol. Cell. Probes* 8:91-98). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene. As used herein, the terms "nucleic acid," "nucleic acid molecule," or "polynucleotide" are intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof.

[00110] The term "expression" refers to the biosynthesis of a product encoded by a nucleic acid. For example, in the case of nucleic acid segment encoding a polypeptide of interest, expression involves transcription of the nucleic acid segment into mRNA and the translation of mRNA into one or more polypeptides.

[00111] The terms "expression unit" and "expression cassette" are used interchangeably herein and denote a nucleic acid segment encoding a polypeptide of interest and capable of providing expression of the nucleic acid segment in a host cell. An expression unit

typically comprises a transcription promoter, an open reading frame encoding the polypeptide of interest, and a transcription terminator, all in operable configuration. In addition to a transcriptional promoter and terminator, an expression unit can further include other nucleic acid segments such as, *e.g.*, an enhancer or a polyadenylation signal.

[00112] The term “expression vector,” as used herein, refers to a nucleic acid molecule, linear or circular, comprising one or more expression units. In addition to one or more expression units, an expression vector can also include additional nucleic acid segments such as, for example, one or more origins of replication or one or more selectable markers. Expression vectors are generally derived from plasmid or viral DNA, or can contain elements of both.

[00113] As used herein, a “polypeptide,” “polypeptide chain,” or “protein” refers to a single, linear and contiguous arrangement of covalently linked amino acids. Polypeptides can form one or more intrachain disulfide bonds. With regard to polypeptides as described herein, reference to modifications or alterations of amino acid residues corresponding to those specified by SEQ ID NO includes post-translational modifications of such residues. The terms polypeptide and protein also encompass embodiments where two polypeptide chains link together in a non-linear fashion, such as via an interchain disulfide bond. For example, a native immunoglobulin molecule is comprised of two heavy chain polypeptides and two light chain polypeptides. Each of the heavy chain polypeptides associate with a light chain polypeptide by virtue of interchain disulfide bonds between the heavy and light chain polypeptides to form two heterodimeric proteins or polypeptides (*i.e.*, a protein comprised of two heterologous polypeptide chains). The two heterodimeric proteins then associate by virtue of additional interchain disulfide bonds between the heavy chain polypeptides to form an immunoglobulin protein or polypeptide. Herein, a protein or polypeptide may be an antibody or an antigen-binding fragment of an antibody. In some embodiments, a protein may be an scFv-Fc-scFv protein, scFv-scFv dimer, or a diabody.

[00114] As will be appreciated by one of skill in the art, proteins and polypeptides are defined herein in terms of the amino acid sequences of the individual polypeptide chains, which are indicated by the SEQ ID NOs reference throughout this disclosure. For example, in some embodiments an scFv-Fc-scFv protein or polypeptide described herein is comprised of two scFv-Fc-scFv polypeptide chains associated by interchain bonds (*e.g.*, interchain disulfide bonds) to form a dimeric scFv-Fc-scFv protein (*e.g.*, a homodimeric or heterodimeric scFv-Fc-scFv protein) (See, for example, FIG. 10). In such embodiments, the scFv-Fc-scFv protein is defined by the

amino acid sequences of the individual scFv-Fc-scFv polypeptide chains. Polypeptides and proteins can also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents can be added to a protein or polypeptide by the cell in which the protein is produced, and will vary with the type of cell. Proteins and polypeptides are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

[00115] The terms “amino-terminal” and “carboxyl-terminal” are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl-terminus of the reference sequence, but is not necessarily at the carboxyl-terminus of the complete polypeptide.

[00116] As used herein, the term "transformation," "transfection," and "transduction" refer to the transfer of nucleic acid (i.e., a nucleotide polymer) into a cell. As used herein, the term "genetic transformation" refers to the transfer and incorporation of DNA, especially recombinant DNA, into a cell. The transferred nucleic acid can be introduced into a cell via an expression vector.

[00117] "Antibody-dependent cell-mediated cytotoxicity" and "ADCC," as used herein, refer to a cell-mediated process in which nonspecific cytotoxic cells that express FcγRs (*e.g.*, monocytic cells such as natural killer (NK) cells and macrophages) recognize bound antibody (or other protein capable of binding FcγRs) on a target cell and subsequently cause lysis of the target cell. In principle, any effector cell with an activating FcγR can be triggered to mediate ADCC. The primary cells for mediating ADCC are NK cells, which express only FcγRIII, whereas monocytes, depending on their state of activation, localization, or differentiation, can express FcγRI, FcγRII, and FcγRIII. For a review of FcγR expression on hematopoietic cells, see, *e.g.*, Ravetch et al., 1991, *Annu. Rev. Immunol.*, 9:457-92.

[00118] The term “having ADCC activity,” as used herein in reference to a polypeptide or protein, means that the polypeptide or protein, for example, one comprising an Fc domain (*e.g.*, an immunoglobulin hinge region and an immunoglobulin constant region having CH2 and CH3 domains) such as derived from IgG (*e.g.*, IgG1), is capable of mediating antibody-dependent cell-mediated cytotoxicity (ADCC) through binding of a cytolytic Fc receptor (*e.g.*,

Fc γ RIII) on a cytolytic immune effector cell expressing the Fc receptor (*e.g.*, an NK cell). In some embodiments, a multispecific polypeptide or protein (*e.g.*, an anti-5T4 x anti-4-1BB molecule as described herein) comprising an Fc domain may lack effector function (*e.g.*, null ADCC activity) as the result of mutations in the CH2 and/or CH3 domain.

[00119] "Complement-dependent cytotoxicity" and "CDC," as used herein, refer to a process in which components in normal serum ("complement"), together with an antibody or other C1q-complement-binding protein bound to a target antigen, exhibit lysis of a target cell expressing the target antigen. Complement consists of a group of serum proteins that act in concert and in an orderly sequence to exert their effect.

[00120] The terms "classical complement pathway" and "classical complement system," as used herein, are synonymous and refer to a particular pathway for the activation of complement. The classical pathway requires antigen-antibody complexes for initiation and involves the activation, in an orderly fashion, of nine major protein components designated C1 through C9. For several steps in the activation process, the product is an enzyme that catalyzes the subsequent step. This cascade provides amplification and activation of large amounts of complement by a relatively small initial signal.

[00121] The term "having CDC activity," as used herein in reference to a polypeptide or protein, means that the polypeptide or protein, for example, one comprising an Fc domain (*e.g.*, an immunoglobulin hinge region and an immunoglobulin constant region having CH2 and CH3 domains) such as derived from IgG (*e.g.*, IgG1) is capable of mediating complement-dependent cytotoxicity (CDC) through binding of C1q complement protein and activation of the classical complement system. In some embodiments, a multispecific polypeptide or protein (*e.g.*, an anti-5T4 x anti-4-1BB molecule as described herein) may have lack effector function (*e.g.*, null CDC activity) as the result of one or more mutations in the CH2 and/or CH3 domains.

[00122] "Enhanced effector cell activation" as used herein, refers to the increase, prolonging, and/or potentiation of an effector cell response by the polypeptides or proteins described herein. In some embodiments, enhanced effector cell activation refers to an increase in the cytotoxic activity of an effector cell. In some embodiments, enhanced effector cell activation refers to an increase in cytokine production, cell proliferation, or a change in cell-surface molecule expression such that the ability of the effector cell to lyse a target cell is enhanced. In certain

embodiments, the polypeptides and proteins described herein enhance effector cell activation by modulating Wnt/ β -catenin signaling.

[00123] As used herein, the term “effector cell” refers to a cell of the immune system that is capable of lysing or killing a target cell, such as a tumor cell. Herein, an effector cell may refer to a lymphocyte, such as a T cell, a natural killer (NK) cell, or an NKT cell, a monocyte, a macrophage, a dendritic cell, or a granulocyte. In particular embodiments, the term effector cell refers to a T cell, an NK cell, or an NKT cell.

[00124] As used herein, the terms “treatment,” “treating,” or “ameliorating” refers to either a therapeutic treatment or prophylactic/preventative treatment. A treatment is therapeutic if at least one symptom of disease in an individual receiving treatment improves or a treatment can delay worsening of a progressive disease in an individual, or prevent onset of additional associated diseases.

[00125] As used herein, the term “therapeutically effective amount (or dose)” or “effective amount (or dose)” of a polypeptide or protein described herein or a composition thereof refers to that amount of the compound sufficient to result in amelioration of one or more symptoms of the disease being treated in a statistically significant manner or a statistically significant improvement in organ function. When referring to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When referring to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered serially or simultaneously (in the same formulation or concurrently in separate formulations).

[00126] Herein, the term “statistical significance” refers the probability of obtaining a test result that occurs by chance. For example, an observation or test result is said to be statistically significant if the probability of it occurring purely by chance (p) is less than the predetermined statistical threshold (α), or $p < \alpha$. The statistical threshold for a particular test may be set according to the characteristics of the data and conventions known in the art. For example, α is conventionally set to 5% (0.05), such that, for a given result, $p < 0.05$ in order for said result to be considered statistically significant.

[00127] As used herein, a “5T4-binding domain” refers to a domain of a polypeptide described herein that specifically bind to oncofetal trophoblast glycoprotein (5T4) (*e.g.*, human 5T4), also known as TPBG and Wnt-activated inhibitory factor 1 (WAIF1). 5T4 is a 72kD

oncofetal glycoprotein that is heavily N-glycosylated proteins with several leucine-rich repeats associated with protein-protein interactions. The term “5T4” may refer to any isoform of 5T4. Exemplary human 5T4 nucleotide sequences are shown in Table 1 below and provided in SEQ ID NOs: 163 and 167 and exemplary human 5T4 amino acid sequences are provided in SEQ ID NOs: 164 and 168.

[00128] As used herein, “4-1BB-binding domain” refers to a binding domain of a protein or polypeptide described herein that is capable of specifically binding to human 4-1BB (also known as CD137). The term “4-1BB” may refer to any isoform of 4-1BB. Exemplary human 4-1BB nucleotide sequences are shown in Table 1 below and provided in SEQ ID NOs: 161 and 165, and exemplary human 4-1BB amino acid sequences are provided in SEQ ID NOs: 162 and 166.

Table 1. Exemplary 4-1BB and 5T4 DNA and amino acid sequences

Protein Name	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
Full length human 4-1BB	ATGGGAAACAGCTGTTACAACATAG TAGCCACTCTGTTGCTGGTCCTCAAC TTTGAGAGGACAAGATCATTGCAGG ATCCTTGTAGTAACTGCCCAGCTGGT ACATTCTGTGATAATAACAGGAATCA GATTTGCAGTCCCTGTCCTCCAAATA GTTTCTCCAGCGCAGGTGGACAAAG GACCTGTGACATATGCAGGCAGTGTA AAGGTGTTTTTCAGGACCAGGAAGGA GTGTTCTCCACCAGCAATGCAGAGT GTGACTGCACTCCAGGGTTTCACTGC CTGGGGGCAGGATGCAGCATGTGTG AACAGGATTGTAAACAAGGTCAAGA ACTGACAAAAAAGGTTGTAAAGAC TGTTGCTTTGGGACATTTAACGATCA GAAACGTGGCATCTGTGCGACCCTGGA CAAACGTGTTCTTTGGATGGAAAGTCT GTGCTTGTGAATGGGACGAAGGAGA GGGACGTGGTCTGTGGACCATCTCCA GCCGACCTCTCTCCGGGAGCATCCTC TGTGACCCCGCCTGCCCCTGCGAGAG AGCCAGGACACTCTCCGCAGATCATC TCCTTCTTTCTTGCGCTGACGTCGACT GCGTTGCTCTTCTGCTGTTCTTCTC	161	MGNSCYNIVATLLL VLNFERTRSLQDPC SNCPAGTFCDNNR NQICSPCPPNSFSSA GGQRTCDICRQCK GVFRTRKECSSTSN AECDCPTPGFHCLG AGCSMCEQDCKQG QELTKKGCKDCCF GTFNDQKRGICRP WTNCSLDGKSVLV NGTKERDVVCGPS PADLSPGASSVTPP APAREPGHSPQIISF FLALTSTALLFLLFF LTLRFSVVKRGRK KLLYIFKQPFMRPV QTTQEEDGCSCRFP EEEEGGCEL	162

	ACGCTCCGTTTCTCTGTTGTTAAACG GGGCAGAAAGAACTCCTGTATATA TTCAAACAACCATTTATGAGACCAGT ACAAACTACTCAAGAGGAAGATGGC TGTAAGCTGCCGATTTCCAGAAGAAGA AGAAGGAGGATGTGAACTGTGA			
Full length human 5T4	ATGCCTGGGGGGTGCTCCCGGGGCCC CGCCGCCGGGGACGGGCGTCTGCGG CTGGCGCGACTAGCGCTGGTACTCCT GGGCTGGGTCTCCTCGTCTTCTCCCA CCTCCTCGGCATCCTCCTTCTCCTCCT CGGCGCCGTTTCTGGCTTCCGCCGTG TCCGCCCAGCCCCCGCTGCCGGACCA GTGCCCCGCGCTGTGCGAGTGCTCCG AGGCAGCGCGCACAGTCAAGTGCGT TAACCGCAATCTGACCGAGGTGCCCA CGGACCTGCCCGCCTACGTGCGCAAC CTCTTCCTTACCGGCAACCAGCTGGC CGTGCTCCCTGCCGGCGCCTTCGCCC GCCGGCCGCCGCTGGCGGAGCTGGC CGCGCTCAACCTCAGCGGCAGCCGCC TGGACGAGGTGCGCGCGGGCGCCTT CGAGCATCTGCCCAGCCTGCGCCAGC TCGACCTCAGCCACAACCCACTGGCC GACCTCAGTCCCTTCGCTTTCTCGGG CAGCAATGCCAGCGTCTCGGCCCCCA GTCCCCTTGTGGAAGTATCCTGAAC CACATCGTGCCCCCTGAAGATGAGCG GCAGAACCGGAGCTTCGAGGGCATG GTGGTGGCGGCCCTGCTGGCGGGCC GTGCACTGCAGGGGCTCCGCCGCTTG GAGCTGGCCAGCAACCACTTCCTTTA CCTGCCGCGGGATGTGCTGGCCCAAC TGCCAGCCTCAGGCACCTGGACTTA AGTAATAATTGCTGGTGAGCCTGAC CTACGTGTCCTTCCGCAACCTGACAC ATCTAGAAAGCCTCCACCTGGAGGA CAATGCCCTCAAGGTCCTTCACAATG GCACCCTGGCTGAGTTGCAAGGTCTA CCCCACATTAGGGTTTTCTTGACAA CAATCCCTGGGTCTGCGACTGCCACA TGGCAGACATGGTGACCTGGCTCAA GGAAACAGAGGTAGTGAGGGCAAA GACCGGCTCACCTGTGCATATCCGGA AAAAATGAGGAATCGGGTCCTCTTG GAACTCAACAGTGCTGACCTGGACTG	163	MPGGCSRGPAAAGD GRLRLARLALVLL GWVSSSSPTSSASS FSSSAPFLASAVSA QPPLPDQCPALCEC SEAARTVKCVNRN LTEVPTDLPAYVRN LFLTGNQLAVLPAG AFARRPPLAELAAL NLSGSRLDEVVRAG AFEHLPSLRQLDLS HNPLADLSPFAFSG SNASVSAPSPLVELI LNHIVPPEDERQNR SFEGMVVAALLAG RALQGLRRLELASN HFLYLPRDVLAQLP SLRHLDSLNNLSVS LTYVSFRNLTHLES LHLEDNALKVLHN GTLAELQGLPHIRV FLDNNPWVCDCHM ADMVTWLKETEVV QGKDRLTCAYPEK MRNRVLELNSAD LDCDPILPPSLQTSY VFLGIVLALIGAIFL LVLYLNRKGIKKW MHNIRDACRDHME GYHYRYEINADPRL TNLSSNSDV	164

	TGACCCGATTCTTCCCCCATCCCTGC AAACCTCTTATGTCTTCCTGGGTATT GTTTTAGCCCTGATAGGCGCTATTTT CCTCCTGGTTTTGTATTTGAACCGCA AGGGGATAAAAAAGTGGATGCATAA CATCAGAGATGCCTGCAGGGATCAC ATGGAAGGGTATCATTACAGATATG AAATCAATGCGGACCCCAGATTAAC GAACCTCAGTTCTAACTCGGATGTCT GA			
human 4-1BB (ECD*)	ATGGGAAACAGCTGTTACAACATAG TAGCCACTCTGTTGCTGGTCCCTCAAC TTTGAGAGGACAAGATCATTGCAGG ATCCTTGTAGTAACTGCCAGCTGGT ACATTCTGTGATAATAACAGGAATCA GATTTGCAGTCCCTGTCCTCCAAATA GTTTCTCCAGCGCAGGTGGACAAAG GACCTGTGACATATGCAGGCAGTGTA AAGGTGTTTTTCAGGACCAGGAAGGA GTGTTCTCCACCAGCAATGCAGAGT GTGACTGCACTCCAGGGTTTCACTGC CTGGGGGCAGGATGCAGCATGTGTG AACAGGATTGTAAACAAGGTCAAGA ACTGACAAAAAAGGTTGTAAAGAC TGTTGCTTTGGGACATTTAACGATCA GAAACGTGGCATCTGTTCGACCCTGGA CAAACCTGTTCTTTGGATGGAAAGTCT GTGCTTGTGAATGGGACGAAGGAGA GGGACGTGGTCTGTGGACCATCTCCA GCCGACCTCTCTCCGGGAGCATCCTC TGTGACCCCGCCTGCCCTGCGAGAG AGCCAGGACACTCTCCGCAG	165	MGNSCYNIVATLLL VLNFERTRSLQDPC SNCPAGTFCDNNR NQICSPCPPNSFSSA GGQRTCDICRQCK GVFRTRKECSSTSN AECDCPTPGFHCLG AGCSMCEQDCKQG QELTKKGCKDCCF GTFNDQKRGICRP WTNCSLDGKSVLV NGTKERDVVCGPS PADLSPGASSVTPP APAREPGHSPQ	166
human 5T4 (ECD*)	ATGCCTGGGGGGTGCTCCCGGGGCCC CGCCGCCGGGGACGGGCGTCTGCGG CTGGCGCGACTAGCGCTGGTACTCCT GGGCTGGGTCTCCTCGTCTTCTCCCA CCTCCTCGGCATCCTCCTTCTCCTCCT CGGCGCCGTTCTGGCTTCCGCCGTG TCCGCCCAGCCCCCGCTGCCGGACCA GTGCCCCGCGCTGTGCGAGTGCTCCG AGGCAGCGCGCACAGTCAAGTGCGT TAACCGCAATCTGACCGAGGTGCCCA CGGACCTGCCCGCCTACGTGCGCAAC CTCTTCCTTACCGGCAACCAGCTGGC CGTGCTCCCTGCCGGCGCCTTCGCCC GCCGGCCGCCGCTGGCGGAGCTGGC	167	MPGGCSRGPAAAGD GRLRLARLALVLL GWVSSSSPTSSASS FSSSAPFLASAVSA QPPLPDQCPALCEC SEAARTVKCVNRN LTEVPTDLPAYVRN LFLTGNQLAVLPAG AFARRPPLAELAAL NLSGSRLDEVRA AFEHLPSLRQLDLS HNPLADLSPFAFSG SNASVSAPSPLVELI LNHIVPPEDERQNR	168

CGCGCTCAACCTCAGCGGCAGCCGCC TGGACGAGGTGCGCGCGGGCGCCTT CGAGCATCTGCCCAGCCTGCGCCAGC TCGACCTCAGCCACAACCCACTGGCC GACCTCAGTCCCTTCGCTTTCTCGGG CAGCAATGCCAGCGTCTCGGCCCCCA GTCCCCTTGTGGAAGTATCCTGAAC CACATCGTGCCCCCTGAAGATGAGCG GCAGAACCGGAGCTTCGAGGGCATG GTGGTGGCGGCCCTGCTGGCGGGCC GTGCACTGCAGGGGCTCCGCCGCTTG GAGCTGGCCAGCAACCACTTCCTTTA CCTGCCGCGGGATGTGCTGGCCCAAC TGCCCAGCCTCAGGCACCTGGACTTA AGTAATAATTGCTGGTGAGCCTGAC CTACGTGTCCTTCCGCAACCTGACAC ATCTAGAAAGCCTCCACCTGGAGGA CAATGCCCTCAAGGTCCTTCACAATG GCACCCTGGCTGAGTTGCAAGGTCTA CCCCACATTAGGGTTTTCTGGACAA CAATCCCTGGGTCTGCGACTGCCACA TGGCAGACATGGTGACCTGGCTCAA GGAAACAGAGGTAGTGCAGGGCAAA GACCGGCTCACCTGTGCATATCCGGA AAAAATGAGGAATCGGGTCCTCTTG GAACTCAACAGTGCTGACCTGGACTG TGACCCGATTCTTCCCCCATCCCTGC AAACCTCT	SFEGMVVAALLAG RALQGLRRLELASN HFLYLPRDVLAQLP SLRHLDSLNNLSVS LTYVSFRNLTHLES LHLEDNALKVLHN GTLAELQGLPHIRV FLDNNPWVCDCHM ADMVTWLKETEVV QGKDRLTCAYPEK MRNRVLELNSAD LDCDPILPPSLQTS
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*ECD = Extracellular domain

[00129] As used herein, a “multispecific polypeptide” refers to a polypeptide comprising two or more binding domains each capable of specifically binding to a target antigen. For example, the polypeptides described herein may comprise 2, 3, 4, or more binding domains and may be able to bind 2, 3, 4, or more target antigens. In some embodiments, a multispecific polypeptide is a bispecific polypeptide. Herein, a “bispecific polypeptide” comprises two binding domains and capable of binding to two distinct target antigens. In some embodiments, the bispecific polypeptides described herein comprise a first binding domain that specifically binds to a cell surface antigen expressed on a target cell, such as 5T4. In some embodiments, the bispecific polypeptides described herein comprise a binding domain that specifically binds to a cell surface antigen expressed on an effector cell, such as 4-1BB. In particular embodiments, the multispecific polypeptide is an ADAPTIR™ homodimer bispecific polypeptide in the format scFv-Fc-scFv.

[00130] Multispecific polypeptides using scaffolds are disclosed, for instance, in PCT Publication Nos. WO 2007/146968; WO 2010/040105; WO 2010/003108; WO 2016/094873; WO 2017/053469; U.S. Patent Application Publication No. 2006/0051844; and U.S. Patent Nos. 7,166,707; and 8,409,577, which are each incorporated herein by reference in their entirety. In certain embodiments, the multispecific polypeptides described herein are bispecific polypeptides and may comprise an scFv-Fc-scFv structure, also referred to herein as an ADAPTIR™ polypeptide, or Format 1, an exemplary embodiment of which is shown in FIG. 10. The structure of a polypeptide comprising a Format 1 structure comprises, from N-terminus to C-terminus: a first scFv binding domain – an immunoglobulin (Ig) hinge region – an Ig constant region – a second scFv binding domain (FIG. 10). In certain embodiments, the multispecific polypeptides described herein may comprise an IgG-scFv structure (also referred to herein as the Morrison format, or Format 2). The structure of a polypeptide comprising a Format 2 structure comprises, from N-terminus to C-terminus: an scFv binding domain – an Ig constant region – an Ig hinge region – an Ig variable region. Format 2 is, essentially, an intact Ig molecule comprising a C-terminal scFv domain.

Binding polypeptides and proteins

[00131] In some embodiments, the present disclosure describes polypeptides capable of specifically binding to 5T4 (*e.g.*, 5T4-binding polypeptides), as well as multispecific polypeptides and proteins comprising these binding domains. Such embodiments may be referred to as 5T4-binding polypeptides. In particular embodiments, the present invention provides multispecific binding proteins comprising a 5T4-binding domain and a second binding domain capable of binding to a cell-surface molecule on an effector cell. In particular embodiments, the present invention provides bispecific binding proteins comprising a 5T4-binding domain and a second binding domain capable of binding to 4-1BB (*e.g.*, a 4-1BB-binding domain). In some embodiments, the 5T4-binding polypeptides comprise the structure, from N-terminus to C-terminus: a 5T4-binding domain – Fc domain.

[00132] In some embodiments, the present disclosure describes polypeptides capable of specifically binding to 4-1BB, as well as multispecific polypeptides and proteins comprising these binding domains. Such embodiments may be referred to as 4-1BB-binding polypeptides. In particular embodiments, the present invention provides multispecific

polypeptides comprising a 4-1BB-binding domain and a second binding domain capable of binding to a cell-surface molecule on a target cell. In particular embodiments, the present invention provides bispecific polypeptides comprising a 4-1BB-binding domain and a second binding domain capable of binding to 5T4 (*e.g.*, a 5T4-binding domain). In some embodiments, the 4-1BB-binding polypeptides comprise the structure, from N-terminus to C-terminus: a 4-1BB-binding domain – Fc domain.

[00133] In some embodiments, the polypeptides described herein can further comprise immunoglobulin constant regions, linker peptides, hinge regions, immunoglobulin dimerization/heterodimerization domains, junctional amino acids, tags, *etc.* These components of the disclosed polypeptides and proteins are described in further detail below.

[00134] The polypeptides and proteins described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) comprise one or more binding domains capable of specifically binding a target antigen. The binding domains described herein can be in the form of an antibody or a fusion protein of any of a variety of different formats (*e.g.*, the fusion protein can be in the form of a bispecific or multispecific molecule). Non-limiting examples of bispecific molecules include an scFv-Fc-scFv molecule (*e.g.* a bispecific protein comprising the structure of Format 1), an scFv-Ig molecule (*e.g.* a bispecific protein comprising the structure of Format 2) and an scFv-scFv molecule. In some embodiments, the bispecific molecules described herein comprise or consist of a first binding domain scFv linked to a second binding domain scFv and do not include other sequences such as an immunoglobulin constant region. In other embodiments, the bispecific protein described herein are diabodies.

[00135] In some embodiments, the polypeptides and proteins described herein are conjugated to a drug or a toxic moiety.

[00136] In some embodiments, the polypeptides and proteins described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) may comprise sequences amino acid and/or nucleic acid shown in Tables 2-10. In certain embodiments, the binding domains of the polypeptides described herein comprise (i) an immunoglobulin light chain variable region (V_L) comprising CDRs LCDR1, LCDR2, and LCDR3, and (ii) an immunoglobulin heavy chain variable region (V_H) comprising CDRs HCDR1, HCDR2, and HCDR3. In some embodiments, amino acid sequences provided for polypeptide constructs do

not include the human immunoglobulin leader sequences. CDR sequences and amino acid substitution positions shown are those defined using the IMGT criteria (Brochet et al, Nucl. Acids Res. (2008) 36, W503-508). In some cases, the sequences shown in the disclosure, including in Tables 2-10, contain amino acid substitutions relative to a parent sequence (*e.g.* an anti-4-1BB antibody or binding fragment thereof such as clone 1618/1619, which is disclosed in PCT Application Publication No. WO 2016/185016, or an anti-5T4 antibody or binding fragment thereof such as those disclosed in PCT Application Publication No. WO 2016/185016). In some embodiments, the parent sequence of an anti-5T4 binding domain comprises the amino acid sequence provided in SEQ ID NOs: 38 and 68. In some embodiments, the parent sequence of an anti-4-1BB binding domain comprises the amino acid sequence provided in SEQ ID NOs: 28 and 16. Exemplary parental sequences for the anti-4-1BB and anti-5T4 binding domains described herein are shown below in Table 2. Underlined text indicates CDR sequences.

Table 2. Exemplary Binding Domain Parental Sequences

Parental Sequence	Amino Acid Sequence	SEQ ID NO:
1618 anti-4-1BB V_H	EVQLLES GGGLVQPGGSLRLSCAASGFTFSYG SMYWVRQAPGKGLEWVSS ISSGSGSTYY ADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARSSYYGSYYSIDYWGQGTLTVVSS	28
1618 anti-4-1BB V_L	DIQMTQSPSSLSASVGDRVTITCRASQSISS YLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQYYDNLPTFGQGTKLEIK	16
1210 anti-5T4 V_H	EVQLLES GGGLVQPGGSLRLSCAASGFTFSS YAMSWVRQAPGKGLEWVSAI SGSGSTYY ADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDYWGQGTLTVVSS	38
1210 anti-5T4 V_L	DIQMTQSPSSLSASVGDRVTITCRASQSISS YLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQTYGYLHTFGQGTKLEIK	68

[00137] In certain embodiments, a binding domain V_L and/or V_H region of the present disclosure is derived from a V_L and/or V_H of a parent V_L and/or V_H region (*e.g.*, 1618/1619 as described in PCT Application Publication No. WO 2016/185016) and optionally contains about one or more (*e.g.*, about 2, 3, 4, 5, 6, 7, 8, 9, 10) insertions, about one or more (*e.g.*, about 2, 3, 4, 5, 6, 7, 8, 9, 10) deletions, about one or more (*e.g.*, about 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions (*e.g.*, conservative amino acid substitutions or non-conservative amino acid substitutions), or a combination of the above-noted changes, when compared to the V_L and/or V_H

sequence of a known monoclonal antibody. The insertion(s), deletion(s) or substitution(s) can be anywhere in the V_L and/or V_H region, including at the amino- or carboxyl-terminus or both ends of this region, provided that each CDR comprises zero changes or at most one, two, or three changes. In some embodiments, the binding domain containing the modified V_L and/or V_H region can still specifically bind its target with an affinity similar to or greater than the parent binding domain.

[00138] In some embodiments, a binding domain described herein is derived from an antibody and comprises a variable heavy chain (V_H) and a variable light chain (V_L). For example, an scFv comprising a V_H and V_L chain. These binding domains and variable chains may be arranged in any order that still retains some binding to the target(s). For example, the variable domains may be arranged in the order such as V_H 5T4 – V_L 5T4 – V_H 4-1BB – V_L 4-1BB; V_L 5T4 – V_H 5T4 – V_H 4-1BB – V_L 4-1BB; V_H 5T4 – V_L 5T4 – V_L 4-1BB – V_H 4-1BB; V_L 5T4 – V_H 5T4 – V_L 4-1BB – V_H 4-1BB; V_H 4-1BB – V_L 4-1BB – V_H 5T4 – V_L 5T4; V_L 4-1BB – V_H 4-1BB – V_L 5T4 – V_H 5T4; V_H 4-1BB – V_L 4-1BB – V_L 5T4 – V_H 5T4; or V_L 4-1BB – V_H 4-1BB – V_H 5T4 – V_L 5T4. The pairs of V_H regions and V_L regions in the binding domain binding to 4-1BB and/or the binding domain binding to 5T4 may be in the format of a single chain antibody (scFv).

[00139] In some embodiments, the polypeptides and proteins described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) comprise binding domains that are scFvs. In such embodiments, the binding domains may be referred to as scFv domains. In some embodiments, a binding domain is a single-chain Fv fragment (scFv) that comprises V_H and V_L regions specific for a target of interest. In certain embodiments, the V_H and V_L regions are human or humanized. In some variations, a binding domain is a single-chain Fv (scFv) comprising V_L and V_H regions joined by a peptide linker.

[00140] The use of peptide linkers for joining V_L and V_H regions is well-known in the art, and a large number of publications exist within this particular field. In some embodiments, a peptide linker is a 15mer consisting of three repeats of a Gly-Gly-Gly-Gly-Ser amino acid sequence ((Gly₄Ser)₃) (SEQ ID NO: 96). Other linkers have been used, and phage display technology, as well as selective infective phage technology, has been used to diversify and select appropriate linker sequences (Tang *et al.*, *J. Biol. Chem.* 271, 15682-15686, 1996; Hennecke *et al.*, *Protein Eng.* 11, 405-410, 1998). In certain embodiments, the V_L and V_H regions are joined by a peptide linker having an amino acid sequence comprising the formula (Gly₄Ser)_n, wherein n =

1-5. For instance, in one embodiment of the invention, the linker comprises (Gly₄Ser)₄. Other suitable linkers can be obtained by optimizing a simple linker (*e.g.*, (Gly₄Ser)_n) through random mutagenesis. In some embodiments, the V_H region of the scFv described herein may be positioned N-terminally to a linker sequence. In some embodiments, the V_L region of the scFvs described herein may be positioned C-terminally to the linker sequence. In some embodiments, the scFv may bind to 5T4 and/or 4-1BB more effectively than the antibody comprising the same V_H and V_L region sequences in the same orientation. In certain embodiments, the scFv may bind more effectively to 5T4 and/or 4-1BB in the V_L-V_H orientation than in the V_H-V_L orientation, or vice versa.

[00141] In some embodiments, the polypeptide comprises a binding domain linker linking the binding domains (*e.g.*, linking the scFv domains). In some embodiments, the binding domain linker is a Gly₄Ser linker. In some embodiments, the binding domain linker is a 20mer consisting of four repeats of a Gly-Gly-Gly-Gly-Ser amino acid sequence ((Gly₄Ser)₃) (SEQ ID NO: 98). In some embodiments, the binding domain linker comprises an amino acid sequence selected from SEQ ID NOs 86-108. Other linkers have been used, and phage display technology, as well as selective infective phage technology, has been used to diversify and select appropriate linker sequences (Tang *et al.*, *J. Biol. Chem.* 271, 15682-15686, 1996; Hennecke *et al.*, *Protein Eng.* 11, 405-410, 1998). In certain embodiments, the V_L and V_H regions are joined by a peptide linker having an amino acid sequence comprising the formula (Gly₄Ser)_n, wherein n = 1-5. Other suitable linkers can be obtained by optimizing a simple linker (*e.g.*, (Gly₄Ser)_n) through random mutagenesis. In some instances, a bispecific molecule may comprise an scFv binding to 5T4 linked to an scFv binding to 4-1BB. In some embodiments, bispecific molecules do not comprise a hinge region or a constant region.

[00142] In some embodiments, the polypeptides and proteins described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) further comprise a hinge. In some embodiments, the hinge is an altered immunoglobulin hinge in which one or more cysteine residues in a wild type immunoglobulin hinge region is substituted with one or more other amino acid residues (*e.g.*, serine or alanine). Exemplary altered immunoglobulin hinges, carboxyl-terminus linkers, and amino-terminus linkers include an immunoglobulin human IgG1 hinge region having one, two or three cysteine residues found in a wild type human IgG1 hinge substituted by one, two or three different amino acid residues (*e.g.*,

serine or alanine). An altered immunoglobulin hinge can additionally have a proline substituted with another amino acid (*e.g.*, serine or alanine). For example, the above-described altered human IgG1 hinge can additionally have a proline located carboxyl-terminal to the three cysteines of wild type human IgG1 hinge region substituted by another amino acid residue (*e.g.*, serine, alanine). In one embodiment, the prolines of the core hinge region are not substituted. In certain embodiments, a hinge, a carboxyl-terminus linker, or an amino-terminus linker polypeptide comprises or is a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a wild type immunoglobulin hinge region, such as a wild type human IgG1 hinge, a wild type human IgG2 hinge, or a wild type human IgG4 hinge.

[00143] In certain embodiments, a hinge present in a polypeptide that forms a heterodimer with another polypeptide chain can be an immunoglobulin hinge, such as a wild-type immunoglobulin hinge region or an altered immunoglobulin hinge region thereof. In certain embodiments, a hinge of one polypeptide chain of a heterodimeric protein is identical to a corresponding hinge of the other polypeptide chain of the heterodimer. In certain other embodiments, a hinge of one chain is different from that of the other chain (in their length or sequence). The different hinges in the different chains allow different manipulation of the binding affinities of the binding domains to which the hinges are connected, so that the heterodimer is able to preferentially bind to the target of one binding domain over the target of the other binding domain. For example, in certain embodiments, a heterodimeric protein has a 4-1BB-binding domain in one chain and a 5T4-binding domain in another chain. Having two different hinges in the two chains may allow the heterodimer to bind to the 5T4 first, and then to 4-1BB second. Thus, the heterodimer may recruit 4-1BB-expressing effector cells to 5T4-expressing target cells (*e.g.*, 5T4-expressing tumor or cancer cells), which in turn may damage or destroy the 5T4-expressing cells.

[00144] In certain embodiments, a carboxyl-terminus linker or an amino-terminus linker is a flexible linker sequence comprising glycine-serine (*e.g.*, Gly₄Ser) repeats. In certain embodiments, the linker comprises three Gly₄Ser repeats (SEQ ID NO: 96) followed by a proline residue. In certain embodiments the proline residue is followed by an amino acid selected from the group consisting of glycine, arginine and serine. In some embodiments, a carboxyl-terminus linker

or an amino-terminus linker comprises or consists of a sequence selected from SEQ ID NO: 86 - 108.

[00145] Some exemplary hinge, carboxyl-terminus linker, and amino-terminus linker sequences suitable for use in accordance with the present disclosure are Table 3 below. Additional exemplary hinge and linker regions are set forth in SEQ ID NOs: 241-244, 601, 78, 763-791, 228, 379-434, 618-749 of U.S. Patent Publication No. 2013/0129723 (said sequences incorporated by reference herein).

Table 3. Exemplary hinges and linkers

Hinge Region	Amino Acid Sequence	SEQ ID
sss(s)-hIgG1 hinge	EPKSSDKTHTSPPSS	71
csc(s)-hIgG1 hinge	EPKSCDKTHTSPPCS	72
ssc(s)-hIgG1 hinge	EPKSSDKTHTSPPCS	73
scc(s)-hIgG1 hinge	EPKSSDKTHTCPPCS	74
css(s)-hIgG1 hinge	EPKSCDKTHTSPPSS	75
scs(s)-hIgG1 hinge	EPKSSDKTHTCPPSS	76
ccc(s)-hIgG1 hinge	EPKSCDKTHTSPPCS	77
ccc(p)-hIgG1 hinge	EPKSCDKTHTSPPCP	78
sss(p)-hIgG1 hinge	EPKSSDKTHTSPPSP	79
csc(p)-hIgG1 hinge	EPKSCDKTHTSPPCP	80
ssc(p)-hIgG1 hinge	EPKSSDKTHTSPPCP	81
scc(p)-hIgG1 hinge	EPKSSDKTHTCPPCP	82
css(p)-hIgG1 hinge	EPKSCDKTHTSPPSP	83
scs(p)-hIgG1 hinge	EPKSSDKTHTCPPSP	84
Scppcp	SCPPCP	85
STD1	NYGGGGSGGGGSGGGGSGNS	86
STD2	NYGGGGSGGGGSGGGGSGNYGGGGSGG GGSGGGGSGNS	87
H1	NS	88
H2	GGGGSGNS	89
H3	NYGGGGSGNS	90
H4	GGGGSGGGGSGNS	91
H5	NYGGGGSGGGGSGNS	92
H6	GGGGSGGGGSGGGGSGNS	93
H7	GCPPCPNS	94
Gly ₄ Ser	GGGGS	95
(G ₄ S) ₃	GGGGSGGGGSGGGGS	96
H105	SGGGGSGGGGSGGGGS	97
(G ₄ S) ₄	GGGGSGGGGSGGGGSGGGGS	98

Hinge Region	Amino Acid Sequence	SEQ ID
H75 (NKG2A quadruple mutant)	QRHNNSSLNTGTQMAGHSPNS	99
H83 (NKG2A derived)	SSLNTGTQMAGHSPNS	100
H106 (NKG2A derived)	QRHNNSSLNTGTQMAGHS	101
H81 (NKG2D derived)	EVQIPLTESYSPNS	102
H91 (NKG2D derived)	NSLANQEVQIPLTESYSPNS	103
H94	SGGGGSGGGGSGGGGSPNS	104
H111	SGGGGSGGGGSGGGGSPGS	105
H113	SGGGGSGGGGSGGGGSPAS	106
H114	SGGGGSGGGGSGGGGSPS	107
H115	SGGGGSGGGGSGGGGSPSS	108

[00146] In other embodiments, the polypeptides and proteins described herein include a heterodimerization domain that is capable of heterodimerization with a different heterodimerization domain in a second, non-identical polypeptide chain. In certain variations, the second polypeptide chain for heterodimerization includes a second binding domain. Accordingly, in certain embodiments of the present disclosure, two non-identical polypeptide chains, one comprising the polypeptide comprising a first binding domain and the second optionally comprising a second binding domain, dimerize to form a heterodimeric binding protein. Dimerization/heterodimerization domains can be used where it is desired to form heterodimers from two non-identical polypeptide chains, where one or both polypeptide chains comprise a binding domain. In certain embodiments, one polypeptide chain member of certain heterodimers described herein does not contain a binding domain. Examples of types of heterodimers include those described in U.S. Patent Application Publication Nos. 2013/0095097 and 2013/0129723, and International PCT Publication No. WO 2016/094873.

[00147] In certain embodiments, the first and second polypeptide chains dimerize via the inclusion of an “immunoglobulin dimerization domain” or “immunoglobulin heterodimerization domain.” An “immunoglobulin dimerization domain” or “immunoglobulin heterodimerization domain” refers herein to an immunoglobulin domain of a first polypeptide chain that preferentially interacts or associates with a different immunoglobulin domain of a second polypeptide chain, wherein the interaction of the different immunoglobulin domains substantially contributes to or efficiently promotes heterodimerization of the first and second polypeptide chains (*i.e.*, the formation of a dimer between two different polypeptide chains, which

is also referred to as a “heterodimer”). The immunoglobulin heterodimerization domains in the polypeptide chains of a heterodimer are different from each other and thus can be differentially modified to facilitate heterodimerization of both chains and to minimize homodimerization of either chain. Immunoglobulin heterodimerization domains provided herein allow for efficient heterodimerization between different polypeptides and facilitate purification of the resulting heterodimeric protein.

[00148] As provided herein, immunoglobulin heterodimerization domains useful for promoting heterodimerization of two different polypeptide chains according to the present disclosure include wild-type and altered immunoglobulin CH1 and CL domains, for instance, human CH1 and CL domains. In certain embodiments, an immunoglobulin heterodimerization domain is a wild-type CH1 domain, such as a wild type IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM CH1 domain, for example, as set forth in SEQ ID NOs: 114, 186-192 and 194, respectively, of U.S. Patent Application Publication No. 2013/0129723 or SEQ ID NO: 114 of U.S. Patent Application Publication No. 2013/0129723 (said sequence incorporated by reference herein). In further embodiments, a cysteine residue of a wild-type CH1 domain (*e.g.*, a human CH1) involved in forming a disulfide bond with a wild type immunoglobulin CL domain (*e.g.*, a human CL) is deleted or substituted in the altered immunoglobulin CH1 domain such that a disulfide bond is not formed between the altered CH1 domain and the wild-type CL domain.

[00149] In certain embodiments, an immunoglobulin heterodimerization domain is a wild-type CL domain, such as a wild type C κ domain or a wild type C λ domain, for example, as set forth in SEQ ID NOs: 112 and 113, respectively, of U.S. Patent Application Publication No. 2013/0129723 (said sequences incorporated by reference herein). In further embodiments, an immunoglobulin heterodimerization domain is an altered immunoglobulin CL domain, such as an altered C κ or C λ domain, for instance, an altered human C κ or human C λ domain. In certain embodiments, a cysteine residue of a wild-type CL domain involved in forming a disulfide bond with a wild type immunoglobulin CH1 domain is deleted or substituted in the altered immunoglobulin CL domain, for example a C κ domain as set forth in SEQ ID NO: 141 of U.S. Patent Application Publication No. 2013/0129723 or a C λ domain as set forth in SEQ ID NO: 140 of U.S. Patent Application Publication No. 2013/0129723 (said sequences incorporated by reference herein). In certain embodiments, only the last cysteine of the wild type human C κ domain is deleted in the altered C κ domain because the first arginine deleted from the wild type human C κ

domain can be provided by a linker that has an arginine at its carboxyl-terminus and links the amino-terminus of the altered C κ domain with another domain (*e.g.*, an immunoglobulin sub-region, such as a sub-region comprising immunoglobulin CH2 and CH3 domains).

[00150] In further embodiments, an immunoglobulin heterodimerization domain is an altered C κ domain that contains one or more amino acid substitutions, as compared to a wild type C κ domain, at positions that may be involved in forming the interchain-hydrogen bond network at a C κ -C κ interface. For example, in certain embodiments, an immunoglobulin heterodimerization domain is an altered human C κ domain having one or more amino acids at positions N29, N30, Q52, V55, T56, S68 or T70 that are substituted with a different amino acid. The numbering of the amino acids is based on their positions in the altered human C κ sequence as set forth in SEQ ID NO: 141 of U.S. Patent Application Publication No. 2013/0129723 (said sequence incorporated by reference herein). In certain embodiments, an immunoglobulin heterodimerization domain is an altered human C κ domain having one, two, three or four amino acid substitutions at positions N29, N30, V55, or T70. The amino acid used as a substitute at the above-noted positions can be an alanine, or an amino acid residue with a bulk side chain moiety such as arginine, tryptophan, tyrosine, glutamate, glutamine, lysine, aspartate, methionine, serine or phenylalanine. Altered human C κ domains are those that facilitate heterodimerization with a CH1 domain, but minimize homodimerization with another C κ domain. Representative altered human C κ domains are set forth in SEQ ID NOs: 142-178 of U.S. Patent Application Publication No. 2013/0129723; SEQ ID NOs: 160 (N29W V55A T70A), 161 (N29Y V55A T70A), 202 (T70E N29A N30A V55A), 167 (N30R V55A T70A), 168 (N30K V55A T70A), 170 (N30E V55A T70A), 172 (V55R N29A N30A), 175 (N29W N30Y V55A T70E), 176 (N29Y N30Y V55A T70E), 177 (N30E V55A T70E), 178 (N30Y V55A T70E), 838 (N30D V55A T70E), 839 (N30M V55A T70E), 840 (N30S V55A T70E), and 841 (N30F V55A T70E) of U.S. Patent Application Publication No. 2013/0129723 (said sequences incorporated by reference herein).

[00151] In certain embodiments, in addition to or alternative to the mutations in C κ domains described herein, both the immunoglobulin heterodimerization domains (*i.e.*, immunoglobulin CH1 and CL domains) of a polypeptide heterodimer have mutations so that the resulting immunoglobulin heterodimerization domains form salt bridges (*i.e.*, ionic interactions) between the amino acid residues at the mutated sites. For example, the immunoglobulin heterodimerization domains of a polypeptide heterodimer can be a mutated CH1 domain in

combination with a mutated C κ domain. In the mutated CH1 domain, valine at position 68 (V68) of the wild type human CH1 domain is substituted by an amino acid residue having a negative charge (*e.g.*, aspartate or glutamate), whereas leucine at position 29 (L29) of a mutated human C κ domain in which the first arginine and the last cysteine have been deleted is substituted by an amino acid residue having a positive charge (*e.g.*, lysine, arginine or histidine). The charge-charge interaction between the amino acid residue having a negative charge of the resulting mutated CH1 domain and the amino acid residue having a positive charge of the resulting mutated C κ domain forms a salt bridge, which stabilizes the heterodimeric interface between the mutated CH1 and C κ domains. Alternatively, V68 of the wild type CH1 can be substituted by an amino acid residue having a positive charge, whereas L29 of a mutated human C κ domain in which the first arginine and the last cysteine have been deleted can be substituted by an amino acid residue having a negative charge. Exemplary mutated CH1 sequences in which V68 is substituted by an amino acid with either a negative or positive charge are set forth in SEQ ID NOs: 844 and 845 of U.S. Patent Application Publication No. 2013/0129723 (said sequences incorporated by reference herein). Exemplary mutated C κ sequences in which L29 is substituted by an amino acid with either a negative or positive charge are set forth in SEQ ID NOs: 842 and 843 of U.S. Patent Application Publication No. 2013/0129723 (said sequences incorporated by reference herein).

[00152] Positions other than V68 of human CH1 domain and L29 of human C κ domain can be substituted with amino acids having opposite charges to produce ionic interactions between the amino acids in addition or alternative to the mutations in V68 of CH1 domain and L29 of C κ domain. Such positions can be identified by any suitable method, including random mutagenesis, analysis of the crystal structure of the CH1-C κ pair to identify amino acid residues at the CH1-C κ interface, and further identifying suitable positions among the amino acid residues at the CH1-C κ interface using a set of criteria (*e.g.*, propensity to engage in ionic interactions, proximity to a potential partner residue, etc.).

[00153] In certain embodiments, polypeptide heterodimers of the present disclosure contain only one pair of immunoglobulin heterodimerization domains. For example, a first chain of a polypeptide heterodimer can comprise a CH1 domain as an immunoglobulin heterodimerization domain, while a second chain can comprise a CL domain (*e.g.*, a C κ or C λ) as an immunoglobulin heterodimerization domain. Alternatively, a first chain can comprise a CL domain (*e.g.*, a C κ or C λ) as an immunoglobulin heterodimerization domain, while a second chain

can comprise a CH1 domain as an immunoglobulin heterodimerization domain. As set forth herein, the immunoglobulin heterodimerization domains of the first and second chains are capable of associating to form a heterodimeric protein of this disclosure.

[00154] In certain other embodiments, heterodimeric proteins of the present disclosure can have two pairs of immunoglobulin heterodimerization domains. For example, a first chain of a heterodimer can comprise two CH1 domains, while a second chain can have two CL domains that associate with the two CH1 domains in the first chain. Alternatively, a first chain can comprise two CL domains, while a second chain can have two CH1 domains that associate with the two CL domains in the first chain. In certain embodiments, a first polypeptide chain comprises a CH1 domain and a CL domain, while a second polypeptide chain comprises a CL domain and a CH1 domain that associate with the CH1 domain and the CL domain, respectively, of the first polypeptide chain.

[00155] In the embodiments where a heterodimeric protein comprises only one heterodimerization pair (*i.e.*, one immunoglobulin heterodimerization domain in each chain), the immunoglobulin heterodimerization domain of each chain can be located amino-terminal to the immunoglobulin constant region of that chain. Alternatively, the immunoglobulin heterodimerization domain in each chain can be located carboxyl-terminal to the immunoglobulin constant region of that chain.

[00156] In the embodiments where a heterodimeric protein comprises two heterodimerization pairs (*i.e.*, two immunoglobulin heterodimerization domains in each chain), both immunoglobulin heterodimerization domains in each chain can be located amino-terminal to the immunoglobulin constant region of that chain. Alternatively, both immunoglobulin heterodimerization domains in each chain can be located carboxyl-terminal to the immunoglobulin constant region of that chain. In further embodiments, one immunoglobulin heterodimerization domain in each chain can be located amino-terminal to the immunoglobulin constant region of that chain, while the other immunoglobulin heterodimerization domain of each chain can be located carboxyl-terminal to the immunoglobulin constant region of that chain. In other words, in those embodiments, the immunoglobulin constant region is interposed between the two immunoglobulin heterodimerization domains of each chain.

[00157] As indicated herein, in some embodiments, the polypeptides and proteins (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding

proteins thereof) of the present disclosure further comprise an immunoglobulin constant region (also referred to herein as a constant region, Fc domain, Fc region, and the like) in a polypeptide chain. In some embodiments, the immunoglobulin constant region comprises an amino acid sequence according to SEQ ID NO: 158, 160, or a variant thereof. The inclusion of an immunoglobulin constant region slows clearance of the polypeptides and proteins of the present invention from circulation after administration to a subject. By mutations or other alterations, an immunoglobulin constant region further enables relatively easy modulation of polypeptide effector functions (*e.g.*, ADCC, ADCP, CDC, complement fixation, and binding to Fc receptors), which can either be increased or decreased depending on the disease being treated, as known in the art and described herein. In certain embodiments, the polypeptides and proteins described herein comprise an immunoglobulin constant region capable of mediating one or more of these effector functions. In other embodiments, one or more of these effector functions are reduced or absent in an immunoglobulin constant region of a polypeptide or protein described herein present disclosure, as compared to a corresponding wild-type immunoglobulin constant region. For example, for dimeric 5T4-binding or 4-1BB-binding polypeptides designed to enhance effector cell activation, such as, *e.g.*, via the inclusion of a 4-1BB-binding domain, an immunoglobulin constant region preferably has reduced or no effector function relative to a corresponding wild-type immunoglobulin constant region.

[00158] An immunoglobulin constant region present in the polypeptides and proteins of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) can comprise or can be derived from part or all of: a CH2 domain, a CH3 domain, a CH4 domain, or any combination thereof. For example, an immunoglobulin constant region can comprise a CH2 domain, a CH3 domain, both CH2 and CH3 domains, both CH3 and CH4 domains, two CH3 domains, a CH4 domain, two CH4 domains, and a CH2 domain and part of a CH3 domain. In certain embodiments, the polypeptides or proteins described herein do not comprise a CH1 domain.

[00159] A polypeptide or protein described herein may comprise a wild type immunoglobulin CH2 domain or an altered immunoglobulin CH2 domain from certain immunoglobulin classes or subclasses (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD) and from various species (including human, mouse, rat, and other mammals). In certain embodiments, a CH2 domain of a polypeptide or a protein described herein is a wild type human immunoglobulin

CH2 domain, such as wild type CH2 domains of human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD, as set forth in SEQ ID NOs: 115, 199-201 and 195-197, respectively, of U.S. Patent Application Publication No. 2013/0129723 (said sequences incorporated by reference herein). In certain embodiments, the CH2 domain is a wild type human IgG1 CH2 domain as set forth in SEQ ID NO: 115 of U.S. Patent Application Publication No. US 2013/0129723 (said sequence incorporated by reference herein).

[00160] In certain embodiments, an altered CH2 region in a polypeptide or a protein of the present disclosure comprises or is a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a wild type immunoglobulin CH2 region, such as the CH2 region of wild type human IgG1, IgG2, or IgG4, or mouse IgG2a (*e.g.*, IGHG2c).

[00161] An altered immunoglobulin CH2 region in a polypeptide or protein of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) can be derived from a CH2 region of various immunoglobulin isotypes, such as IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgD, from various species (including human, mouse, rat, and other mammals). In certain embodiments, an altered immunoglobulin CH2 region in a fusion protein of the present disclosure can be derived from a CH2 region of human IgG1, IgG2 or IgG4, or mouse IgG2a (*e.g.*, IGHG2c), whose sequences are set forth in SEQ ID NOs: 115, 199, 201, and 320 of U.S. Patent Application Publication No. 2013/0129723 (said sequences incorporated by reference herein). In certain embodiments, an altered CH2 domain of a polypeptide or a protein described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) is an altered human IgG1 CH2 domain with mutations known in the art that enhance or reduce immunological activities (*i.e.*, effector functions) such as ADCC, ADCP, CDC, complement fixation, Fc receptor binding, or any combination thereof.

[00162] In certain embodiments, a CH2 domain of a polypeptide or a protein described herein is an altered immunoglobulin CH2 region (*e.g.*, an altered human IgG1 CH2 domain) that comprises one or more amino acid deletions or substitutions. In some embodiments, the CH2 domain comprises an amino acid substitution at the asparagine of position 297 (*e.g.*, asparagine to alanine). Such an amino acid substitution reduces or eliminates glycosylation at this site and abrogates efficient Fc binding to FcγR and C1q. The sequence of an altered human IgG1

CH2 domain with an Asn to Ala substitution at position 297 is set forth in SEQ ID NO: 324 of U.S. Patent Application Publication No. 2013/0129723 (said sequence incorporated by reference herein). In some embodiments, the altered CH2 domain comprises at least one substitution or deletion at positions 234 to 238. For example, an immunoglobulin CH2 region can comprise a substitution at position 234, 235, 236, 237 or 238; positions 234 and 235; positions 234 and 236; positions 234 and 237; positions 234 and 238; positions 234-236; positions 234, 235 and 237; positions 234, 236 and 238; positions 234, 235, 237, and 238; positions 236-238; or any other combination of two, three, four, or five amino acids at positions 234-238. In some embodiments, an altered CH2 region comprises one or more (*e.g.*, two, three, four or five) amino acid deletions at positions 234-238, for instance, at one of position 236 or position 237 while the other position is substituted. In certain embodiments, the amino acid residues at one or more of positions 234-238 has been replaced with one or more alanine residues. In further embodiments, only one of the amino acid residues at positions 234-238 have been deleted while one or more of the remaining amino acids at positions 234-238 can be substituted with another amino acid (*e.g.*, alanine or serine).

[00163] In some embodiments, the above-noted mutation(s) decrease or eliminate the ADCC activity or Fc receptor-binding capability of a polypeptide that comprises the altered CH2 domain.

[00164] In certain other embodiments, a CH2 domain of a polypeptide or a protein described herein is an altered immunoglobulin CH2 region (*e.g.*, an altered human IgG1 CH2 domain) that comprises one or more amino acid substitutions at positions 253, 310, 318, 320, 322, and 331. For example, an immunoglobulin CH2 region can comprise a substitution at position 253, 310, 318, 320, 322, or 331, positions 318 and 320, positions 318 and 322, positions 318, 320 and 322, or any other combination of two, three, four, five or six amino acids at positions 253, 310, 318, 320, 322, and 331. In such embodiments, the above-noted mutation(s) decrease or eliminate the CDC activity of a polypeptide comprising the altered CH2 domain.

[00165] In certain other embodiments, in addition to the amino acid substitution at position 297, an altered CH2 region of a polypeptide or a protein described herein (*e.g.*, an altered human IgG1 CH2 domain) can further comprise one or more (*e.g.*, two, three, four, or five) additional substitutions at positions 234-238. For example, an immunoglobulin CH2 region can comprise a substitution at positions 234 and 297, positions 234, 235, and 297, positions 234, 236

and 297, positions 234-236 and 297, positions 234, 235, 237 and 297, positions 234, 236, 238 and 297, positions 234, 235, 237, 238 and 297, positions 236-238 and 297, or any combination of two, three, four, or five amino acids at positions 234-238 in addition to position 297. In addition or alternatively, an altered CH2 region can comprise one or more (*e.g.*, two, three, four or five) amino acid deletions at positions 234-238, such as at position 236 or position 237. The additional mutation(s) decreases or eliminates the ADCC activity or Fc receptor-binding capability of a polypeptide comprising the altered CH2 domain. In certain embodiments, the amino acid residues at one or more of positions 234-238 have been replaced with one or more alanine residues. In further embodiments, only one of the amino acid residues at positions 234-238 has been deleted while one or more of the remaining amino acids at positions 234-238 can be substituted with another amino acid (*e.g.*, alanine or serine).

[00166] In certain embodiments, in addition to one or more (*e.g.*, 2, 3, 4, or 5) amino acid substitutions at positions 234-238, a mutated CH2 region of a polypeptide or a protein described herein (*e.g.*, an altered human IgG1 CH2 domain) in a fusion protein of the present disclosure can contain one or more (*e.g.*, 2, 3, 4, 5, or 6) additional amino acid substitutions (*e.g.*, substituted with alanine) at one or more positions involved in complement fixation (*e.g.*, at positions I253, H310, E318, K320, K322, or P331). Examples of mutated immunoglobulin CH2 regions include human IgG1, IgG2, IgG4 and mouse IgG2a CH2 regions with alanine substitutions at positions 234, 235, 237 (if present), 318, 320 and 322. An exemplary mutated immunoglobulin CH2 region is mouse IGHG2c CH2 region with alanine substitutions at L234, L235, G237, E318, K320, and K322.

[00167] In still further embodiments, in addition to the amino acid substitution at position 297 and the additional deletion(s) or substitution(s) at positions 234-238, an altered CH2 region of a polypeptide or a protein described herein (*e.g.*, an altered human IgG1 CH2 domain) can further comprise one or more (*e.g.*, two, three, four, five, or six) additional substitutions at positions 253, 310, 318, 320, 322, and 331. For example, an immunoglobulin CH2 region can comprise a (1) substitution at position 297, (2) one or more substitutions or deletions or a combination thereof at positions 234-238, and one or more (*e.g.*, 2, 3, 4, 5, or 6) amino acid substitutions at positions I253, H310, E318, K320, K322, and P331, such as one, two, three substitutions at positions E318, K320 and K322. The amino acids at the above-noted positions can be substituted by alanine or serine.

[00168] In certain embodiments, an immunoglobulin CH2 region of a polypeptide or a protein described herein comprises: (i) an amino acid substitution at the asparagines of position 297 and one amino acid substitution at position 234, 235, 236 or 237; (ii) an amino acid substitution at the asparagine of position 297 and amino acid substitutions at two of positions 234-237; (iii) an amino acid substitution at the asparagine of position 297 and amino acid substitutions at three of positions 234-237; (iv) an amino acid substitution at the asparagine of position 297, amino acid substitutions at positions 234, 235 and 237, and an amino acid deletion at position 236; (v) amino acid substitutions at three of positions 234-237 and amino acid substitutions at positions 318, 320 and 322; or (vi) amino acid substitutions at three of positions 234-237, an amino acid deletion at position 236, and amino acid substitutions at positions 318, 320 and 322.

[00169] Exemplary altered immunoglobulin CH2 regions with amino acid substitutions at the asparagine of position 297 include: human IgG1 CH2 region with alanine substitutions at L234, L235, G237 and N297 and a deletion at G236 (SEQ ID NO: 325 of U.S. Patent Application Publication No. 2013/0129723, said sequence incorporated by reference herein), human IgG2 CH2 region with alanine substitutions at V234, G236, and N297 (SEQ ID NO: 326 of U.S. Patent Application Publication No. 2013/0129723, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at F234, L235, G237 and N297 and a deletion of G236 (SEQ ID NO: 322 of U.S. Patent Application Publication No. 2013/0129723, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at F234 and N297 (SEQ ID NO: 343 of U.S. Patent Application Publication No. US 2013/0129723, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at L235 and N297 (SEQ ID NO: 344 of U.S. Patent Application Publication No. 2013/0129723, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at G236 and N297 (SEQ ID NO: 345 of U.S. Patent Application Publication No. 2013/0129723, said sequence incorporated by reference herein), and human IgG4 CH2 region with alanine substitutions at G237 and N297 (SEQ ID NO: 346 of U.S. Patent Application Publication No. 2013/0129723, said sequence incorporated by reference herein). These CH2 regions can be used in a polypeptide of the present disclosure (*e.g.*, a 5T4-binding polypeptide and/or a bispecific 4-1BB polypeptide).

[00170] In certain embodiments, in addition to the amino acid substitutions described above, an altered CH2 region of a polypeptide or a protein described herein (*e.g.*, an

altered human IgG1 CH2 domain) can contain one or more additional amino acid substitutions at one or more positions other than the above-noted positions. Such amino acid substitutions can be conservative or non-conservative amino acid substitutions. For example, in certain embodiments, P233 can be changed to E233 in an altered IgG2 CH2 region (*see, e.g.*, SEQ ID NO: 326 of U.S. Patent Application Publication No. 2013/0129723, said sequence incorporated by reference herein). In addition or alternatively, in certain embodiments, the altered CH2 region can contain one or more amino acid insertions, deletions, or both. The insertion(s), deletion(s) or substitution(s) can be anywhere in an immunoglobulin CH2 region, such as at the N- or C-terminus of a wild type immunoglobulin CH2 region resulting from linking the CH2 region with another region (*e.g.*, a binding domain or an immunoglobulin heterodimerization domain) via a hinge.

[00171] In certain embodiments, an altered CH2 domain of a polypeptide or protein described herein is a human IgG1 CH2 domain with alanine substitutions at positions 235, 318, 320, and 322 (*i.e.*, a human IgG1 CH2 domain with L235A, E318A, K320A and K322A substitutions) (SEQ ID NO: 595 of U.S. Patent Application Publication No. 2013/0129723, said sequence incorporated by reference herein), and optionally an N297 mutation (*e.g.*, to alanine). In certain other embodiments, an altered CH2 domain is a human IgG1 CH2 domain with alanine substitutions at positions 234, 235, 237, 318, 320 and 322 (*i.e.*, a human IgG1 CH2 domain with L234A, L235A, G237A, E318A, K320A and K322A substitutions) (SEQ ID NO: 596 of U.S. Patent Application Publication No. 2013/0129723, said sequence incorporated by reference herein), and optionally an N297 mutation (*e.g.*, to alanine).

[00172] In some embodiments, an immunoglobulin constant region of a polypeptide or a protein described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) comprises a human IgG1 CH2 domain comprising the substitutions L234A, L235A, G237A, and K322A, according to the EU numbering system.

[00173] The CH3 domain that can form an immunoglobulin constant region of a polypeptide or a protein described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) can be a wild type immunoglobulin CH3 domain or an altered immunoglobulin CH3 domain thereof from certain immunoglobulin classes or subclasses (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, IgM) of various species (including human, mouse, rat, and other mammals). In certain embodiments, a CH3 domain of a polypeptide described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or

multispecific binding proteins thereof) is a wild type human immunoglobulin CH3 domain, such as wild type CH3 domains of human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM as set forth in SEQ ID NOs: 116, 208-210, 204-207, and 212, respectively of U.S. Patent Application Publication No. 2013/0129723 (said sequences incorporated by reference herein). In certain embodiments, the CH3 domain is a wild type human IgG1 CH3 domain as set forth in SEQ ID NO: 116 of U.S. Patent Application Publication No. 2013/0129723 (said sequence incorporated by reference herein).

[00174] In certain embodiments, a CH3 domain of a polypeptide described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) is an altered human immunoglobulin CH3 domain, such as an altered CH3 domain based on or derived from a wild-type CH3 domain of human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM antibodies. For example, an altered CH3 domain can be a human IgG1 CH3 domain with one or two mutations at positions H433 and N434 (positions are numbered according to EU numbering). The mutations in such positions can be involved in complement fixation. In certain other embodiments, an altered CH3 domain of a polypeptide described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) can be a human IgG1 CH3 domain but with one or two amino acid substitutions at position F405 or Y407. The amino acids at such positions are involved in interacting with another CH3 domain. In certain embodiments, an altered CH3 domain of polypeptide described herein can be an altered human IgG1 CH3 domain with its last lysine deleted. The sequence of this altered CH3 domain is set forth in SEQ ID NO: 761 of U.S. Patent Application Publication No. 2013/0129723 (said sequence incorporated by reference herein).

[00175] In certain embodiments, a polypeptide or a protein described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) comprises a CH3 domain that comprises so called “knobs-into-holes” mutations (*see*, Marvin and Zhu, *Acta Pharmacologica Sinica* 26:649-58, 2005; Ridgway *et al.*, *Protein Engineering* 9:617-21, 1966). More specifically, mutations can be introduced into each of the CH3 domains of each polypeptide chain so that the steric complementarity required for CH3/CH3 association obligates these two CH3 domains to pair with each other. For example, a CH3 domain in one single chain polypeptide of a polypeptide heterodimer can contain a T366W mutation (a “knob” mutation, which substitutes a small amino acid with a larger one), and a CH3 domain in

the other single chain polypeptide of the polypeptide heterodimer can contain a Y407A mutation (a “hole” mutation, which substitutes a large amino acid with a smaller one). Other exemplary knobs-into-holes mutations include (1) a T366Y mutation in one CH3 domain and a Y407T in the other CH3 domain, and (2) a T366W mutation in one CH3 domain and T366S, L368A and Y407V mutations in the other CH3 domain.

[00176] The CH4 domain that can form an immunoglobulin constant region a polypeptide or a protein described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) can be a wild type immunoglobulin CH4 domain or an altered immunoglobulin CH4 domain thereof from IgE or IgM molecules. In certain embodiments, the CH4 domain of a polypeptide described herein is a wild type human immunoglobulin CH4 domain, such as wild type CH4 domains of human IgE and IgM molecules as set forth in SEQ ID NOs: 213 and 214, respectively, of U.S. Patent Application Publication No. 2013/0129723 (said sequences incorporated by reference herein). In certain embodiments, a CH4 domain of a polypeptide described herein is an altered human immunoglobulin CH4 domain, such as an altered CH4 domain based on or derived from a CH4 domain of human IgE or IgM molecules, which have mutations that increase or decrease an immunological activity known to be associated with an IgE or IgM Fc region.

[00177] In certain embodiments, an immunoglobulin constant region of a polypeptide or a protein described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) comprises a combination of CH2, CH3 or CH4 domains (*i.e.*, more than one constant region domain selected from CH2, CH3 and CH4). For example, the immunoglobulin constant region can comprise CH2 and CH3 domains or CH3 and CH4 domains. In certain other embodiments, the immunoglobulin constant region can comprise two CH3 domains and no CH2 or CH4 domains (*i.e.*, only two or more CH3). The multiple constant region domains that form an immunoglobulin constant region of the polypeptides described herein can be based on or derived from the same immunoglobulin molecule, or the same class or subclass immunoglobulin molecules. In certain embodiments, the immunoglobulin constant region is an IgG CH2-CH3 (*e.g.*, IgG1 CH2-CH3, IgG2 CH2-CH3, and IgG4 CH2-CH3) and can be a human (*e.g.*, human IgG1, IgG2, and IgG4) CH2CH3. For example, in certain embodiments, the immunoglobulin constant region of a polypeptide described herein comprises (1) wild type human IgG1 CH2 and CH3 domains, (2) human IgG1 CH2 with N297A substitution

(*i.e.*, CH2(N297A)) and wild type human IgG1 CH3, or (3) human IgG1 CH2(N297A) and an altered human IgG1 CH3 with the last lysine deleted. Alternatively, the multiple constant region domains of a polypeptide or a protein described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) can be based on or derived from different immunoglobulin molecules, or different classes or subclasses immunoglobulin molecules. For example, in certain embodiments, an immunoglobulin constant region comprises both human IgM CH3 domain and human IgG1 CH3 domain. The multiple constant region domains that form an immunoglobulin constant region of a polypeptide described herein can be directly linked together or can be linked to each other via one or more (*e.g.*, about 2- about 10) amino acids.

[00178] Exemplary immunoglobulin constant regions that can be used in a polypeptide or a protein described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) are set forth in SEQ ID NOs: 305-309, 321, 323, 341, 342, and 762 of U.S. Patent Application Publication No. 2013/0129723 (said sequences incorporated by reference herein). Further exemplary immunoglobulin constant regions that can be used in a polypeptide or a protein described herein are provided in Table 4 below.

Table 4: Exemplary immunoglobulin constant regions

Name	Amino Acid Sequence	SEQ ID NO:
SS-Fc domain	SSEPKSSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV SVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVY YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPG	158
Delta SS-Fc domain	EPKSSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVY YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPG	160

[00179] In certain embodiments, the immunoglobulin constant regions of each polypeptide chain of a homodimeric or heterodimeric protein described herein (*e.g.*, 5T4-binding

polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) are identical to each other. In certain other embodiments, the immunoglobulin constant region of one polypeptide chain of a heterodimeric protein is different from the immunoglobulin constant region of the other polypeptide chain of the heterodimer. For example, one immunoglobulin constant region of a heterodimeric protein can contain a CH3 domain with a “knob” mutation, whereas the other immunoglobulin constant region of the heterodimeric protein can contain a CH3 domain with a “hole” mutation.

[00180] In some embodiments, the polypeptides of the present disclosure may comprise an immunoglobulin constant region comprising any of the above described mutations and a binding domain comprising one or more amino acid mutations compared to a parental binding domain amino acid sequence. For example, in some embodiments the polypeptides of the present disclosure may comprise an immunoglobulin constant region comprising one or more of the L234A, L235A, G237A, and K322A mutations in the human IgG1 CH2 domain and a 5T4-binding domain comprising a V_H domain selected from the group consisting of SEQ ID NOs: 38 and 46 and a V_L domain selected from the group consisting of SEQ ID NOs: 44, 48, and 50. In some embodiments, the polypeptides of the present disclosure may comprise an immunoglobulin constant region comprising one or more of the L234A, L235A, G237A, and K322A mutations in the human IgG1 CH2 domain and a 41BB-binding domain comprising a V_H domain amino acid sequence of SEQ ID NO: 14 and a V_L domain amino acid sequence of SEQ ID NO: 16. In some embodiments the polypeptides of the present disclosure may comprise an immunoglobulin constant region comprising one or more of the L234A, L235A, G237A, and K322A mutations in the human IgG1 CH2 domain and a 5T4-binding domain comprising a V_H domain selected from the group consisting of SEQ ID NOs: 38 and 46 and a V_L domain selected from the group consisting of SEQ ID NOs: 44, 48, and 50; and a 41BB-binding domain comprising a V_H domain amino acid sequence of SEQ ID NO: 14, and a V_L domain amino acid sequence of SEQ ID NO: 16.

[00181] Polypeptides and proteins described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) may be made using scaffolding as generally disclosed in U.S. Patent Application Publication Nos. 2013/0129723 and 2013/0095097, which are each incorporated herein by reference in their entirety. The polypeptides described herein may comprise two non-identical polypeptide chains, each polypeptide chain

comprising an immunoglobulin heterodimerization domain. The interfacing immunoglobulin heterodimerization domains are different. In one embodiment, the immunoglobulin heterodimerization domain comprises a CH1 domain or a derivative thereof. In another embodiment, the immunoglobulin heterodimerization domain comprises a CL domain or a derivative thereof. In one embodiment, the CL domain is a C κ or C λ isotype or a derivative thereof.

[00182] In some embodiments, polypeptides and proteins described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) may have improved characteristics compared to other 5T4-binding polypeptides or 4-1BB-binding polypeptides. For example, the 5T4-binding polypeptides, 4-1BB-binding polypeptides, or multispecific proteins thereof of the present invention may exhibit a reduced isoelectric point compared to the isoelectric point of a different 5T4-binding polypeptide and/or 4-1BB-binding polypeptide. “Isoelectric point” or “pI” is the pH at which net charge is zero. The isoelectric point of a protein may be measured by any suitable method, *e.g.*, analytical capillary isoelectric focusing chromatography.

[00183] In some embodiments, polypeptides and proteins described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) may bind to 5T4 (*e.g.*, human 5T4) and/or 4-1BB with a higher affinity than a previously known 5T4- and/or 4-1BB-binding domains and/or a parent 5T4- and/or 4-1BB-binding domain or protein. In some embodiments, the dissociation constant of a 5T4- and/or 4-1BB-binding domain or polypeptide may be about 2-5 nM. In certain embodiments, the off rate of a 5T4- and/or 4-1BB-binding domain or polypeptide may be 4- to 10-fold reduced compared to the off rate of a previously known antibody or scFv construct or the parent 5T4- and/or 4-1BB-binding domain or protein.

[00184] In some embodiments, polypeptides and proteins described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) may have a low level of high molecular weight aggregates produced during recombinant expression of the polypeptide or protein. In some embodiments, polypeptides and proteins described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) may exhibit longer stability in human serum, depending on the combination of domains present in the polypeptide or protein.

5T4-binding domains and proteins comprising the same

[00185] In some embodiments, the present invention provides 5T4-binding domains that specifically bind to 5T4 (*e.g.* human 5T4). In some embodiments, the present invention further provides polypeptides comprising a 5T4-binding domain (*e.g.*, 5T4-binding polypeptides). In certain variations, the 5T4-binding polypeptide comprises a hinge region carboxyl-terminal to the 5T4-binding domain, and an immunoglobulin constant region. In further variations, the 5T4-binding polypeptide comprises a carboxyl-terminus binding domain linker carboxyl-terminal to the immunoglobulin constant region, and a second binding domain carboxyl-terminal to the carboxyl-terminus linker. In yet other variations, a 5T4-binding polypeptide comprises a hinge region amino-terminal to the polypeptide comprising the 5T4-binding domain, and an immunoglobulin constant amino-terminal to the hinge region.

[00186] In some embodiments, the 5T4-binding domains described herein binds an epitope located on the extracellular domain of 5T4 (*e.g.*, an epitope comprised within SEQ ID NO: 168). In certain aspects, this epitope is a discontinuous and/or conformational epitope.

[00187] A 5T4-binding domain polypeptide may specifically bind to human 5T4 and comprise a heavy chain CDR1 (HCDR1), HCDR2, HCDR3, light chain CDR1 (LCDR1), LCDR2, and LCDR3, wherein the HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 52, and 60; the HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 62; the HCDR3 comprises an amino acid sequence of SEQ ID NO: 34; the LCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 42, and 54; the LCDR2 comprises an amino acid sequence of SEQ ID NOs: 10; and the LCDR3 comprises an amino acid sequence of SEQ ID NO: 36.

[00188] In particular embodiments, a 5T4-binding domain polypeptide may specifically bind to human 5T4 and comprise an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 30; (b) the HCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 32; (c) the HCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 34; (d) the LCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 8; the LCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 36. In other particular embodiments, a 5T4-binding domain polypeptide specifically binds to human 5T4 and comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein

(a) the HCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 30; (b) the HCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 32; (c) the HCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 34; (d) the LCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 42; (e) the LCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 36. In other particular embodiments, a 5T4-binding domain polypeptide specifically binds to human 5T4 and comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 52; (b) the HCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 32; (c) the HCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 34; (d) the LCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 54; (e) the LCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 36. In other particular embodiments, a 5T4-binding domain polypeptide specifically binds to human 5T4 and comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 60; (b) the HCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 62; (c) the HCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 34; (d) the LCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 54; (e) the LCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 36.

[00189] In certain embodiments, a 5T4-binding domain polypeptide comprises or is a sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence of a light chain variable region (V_L) selected from the group consisting of SEQ ID NOs: 40, 44, 48, 50, 58, 68, and 70, wherein the polypeptide is a multispecific polypeptide in the format scFv-Fc-scFv and comprises a Y to F substitution in the LCDR1 at position 99 of the V_L and/or a F to S substitution in the FR3 at position 148 of the V_L . In certain embodiments, a polypeptide comprising a 5T4-binding domain comprises an amino acid sequence of a light chain variable region (V_L) selected from the group consisting of SEQ ID NOs: 40, 44, 48, 50, 58, 68, and 70. In certain embodiments, a 5T4-binding domain polypeptide comprises an amino acid sequence of a

heavy chain variable region (V_H) selected from the group consisting of SEQ ID NOs 38, 46, 56, and 64.

[00190] In certain embodiments, a 5T4-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 38 and a V_L region comprising the amino acid sequence of SEQ ID NO: 40. In certain embodiments, a 5T4-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 38 and a V_L region comprising the amino acid sequence of SEQ ID NO: 44. In certain embodiments, a 5T4-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 46 and a V_L region comprising the amino acid sequence of SEQ ID NO: 48. In certain embodiments, a 5T4-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 46 and a V_L region comprising the amino acid sequence of SEQ ID NO: 50. In certain embodiments, a 5T4-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 56 and a V_L region comprising the amino acid sequence of SEQ ID NO: 58. In certain embodiments, a 5T4-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 64 and a V_L region comprising the amino acid sequence of SEQ ID NO: 58. In certain embodiments, a 5T4-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 38 and a V_L region comprising the amino acid sequence of SEQ ID NO: 68. In certain embodiments, a 5T4-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 46 and a V_L region comprising the amino acid sequence of SEQ ID NO: 70.

[00191] Exemplary anti-5T4 binding domain sequences are shown in Table 5.

Table 5. 5T4-binding domain polypeptide CDR and variable region amino acid and DNA sequences

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
ALG.AP V-178	HCDR1	GGCTTCACATTCAGCAGCTATGCT	29	GFTFSSYA	30
	HCDR2	ATCTCCGGCAGCGGCGGAAGCACC	31	ISGSGGST	32

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
ALG.AP V-208	HCDR3	GCCAGGTACTATGGCGGCTACTACTCCGCCTGGATGGACTAC	33	ARYYGGYYSAWMDY	34
	LCDR1	CAGTCCATCTCCAGCTAT	7	QSISSY	8
	LCDR2	GCCGCTTCC	9	AAS	10
	LCDR3	CAGCAGACCTATGGCTACCTGCACACC	35	QQTYGYLHT	36
	V _H	GAAGTGCAGCTGCTGGA GTCCGGAGGAGGACTGG TGCAGCCTGGCGGAAGC CTGAGGCTGAGCTGCGC TGCCTCCGGCTTCACATT CAGCAGCTATGCTATGA GCTGGGTGAGGCAAGCC CCTGGAAAGGGCCTGGA GTGGGTGTCCGCTATCTC CGGCAGCGGCGGAAGCA CCTACTACGCTGACTCC GTCAAGGGCAGGTTCAC CATCAGCCGGGACAACA GCAAGAACACCCTGTAC CTGCAGATGAATAGCCT CAGGGCTGAAGACACCG CTGTGTACTACTGCGCC AGGTACTATGGCGGCTA CTACTCCGCCTGGATGG ACTACTGGGGACAGGGC ACACTGGTGACCGTGTC CAGC	37	EVQLLESGGGLVQ PGGSLRLSCAASG FTFSSYAMSWVRQ APGKGLEWVSAIS GSGGSTYYADSVK GRFTISRDN SKNTL YLQMNSLR AEDT AVYYCARYYGGY YSAWMDYWGQG TLVTVSS	38
	V _L	GATATTCAGATGACACA GTCCCCTAGCTCCCTGTC CGCCAGCGTGGGAGATC GGGTGACCATCACCTGC AGGGCCAGCCAGTCCAT CTCCAGCTATTTAACTG GTACCAGCAGAAGCCTG GAAAGGCTCCCAAGCTG CTGATCTACGCCGCTTCC AGCCTCCAGAGCGGCGT GCCTAGCAGGTTCTCCG GCTCCGGAAGCGGAACA GACTTCACCCTGACCATC AGCTCCCTGCAGCCCGA	39	DIQMTQSPSSLSAS VGDRVITCRASQ SISSYLNWYQQKP GKAPKLLIYAASS LQSGVPSRFSGSGS GTDFTLTISSLQPE DSATYYCQQTYG YLHTFGQG TKLEI K	40

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
		GGACTCCGCTACCTACT ACTGCCAGCAGACCTAT GGCTACCTGCACACCTTC GGCCAGGGCACAAGCT GGAGATCAAG			
ALG.AP V-179 ALG.AP V-209 ALG.AP V-222	HCDR1	GGCTTCACATTCAGCAG CTATGCT	29	GFTFSSYA	30
	HCDR2	ATCTCCGGCAGCGGCGG AAGCACC	31	ISGSGGST	32
	HCDR3	GCCAGGTACTATGGCGG CTACTACTCCGCCTGGAT GGACTAC	33	ARYYGGYYSAWM DY	34
	LCDR1	CAGTCCATCTCCAGCTTC	41	QSISSF	42
	LCDR2	GCCGCTTCC	9	AAS	10
	LCDR3	CAGCAGACCTATGGCTA CCTGCACACC	35	QQTYGYLHT	36
	V _H	GAAGTGCAGCTGCTGGA GTCCGGAGGAGGACTGG TGCAGCCTGGCGGAAGC CTGAGGCTGAGCTGCGC TGCCTCCGGCTTCACATT CAGCAGCTATGCTATGA GCTGGGTGAGGCAAGCC CCTGGAAAGGGCCTGGA GTGGGTGTCCGCTATCTC CGGCAGCGGCGGAAGCA CCTACTACGCTGACTCCG TCAAGGGCAGGTTACCC ATCAGCCGGGACAACAG CAAGAACACCCTGTACC TGCAGATGAATAGCCTC AGGGCTGAAGACACCGC TGTGTACTACTGCGCCAG GTACTATGGCGGCTACT ACTCCGCCTGGATGGAC TACTGGGGACAGGGCAC ACTGGTGACCGTGTCCA GC	37	EVQLLESGGGLVQ PGGSLRLSCAASG FTFSSYAMSWVRQ APGKGLEWVSAIS GSGGSTYYADSVK GRFTISRDN SKNTL YLQMNSLR AEDT AVYYCARYYGGY YSAWMDYWGQG TLVTVSS	38
	V _L	GATATTCAGATGACACA GTCCCCTAGCTCCCTGTC CGCCAGCGTGGGAGATC GGGTGACCATCACCTGC AGGGCCAGCCAGTCCAT	43	DIQMTQSPSSLSAS VGDRVITITCRASQ SISSFLN WYQQKP GKAPKLLIYAASS LQSGVPSRFSGSGS	44

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
		CTCCAGCTTCTTAAACTG GTACCAGCAGAAGCCTG GAAAGGCTCCCAAGCTG CTGATCTACGCCGCTTCC AGCCTCCAGAGCGGCGT GCCTAGCAGGTTCTCCG GCTCCGGAAGCGGAACA GACTTCACCCTGACCATC AGCTCCCTGCAGCCCGA GGACTCCGCTACCTACT ACTGCCAGCAGACCTAT GGCTACCTGCACACCTTC GGCCAGGGCACAAAGCT GGAGATCAAG		GTDFTLTISSLQPE DSATYYCQQTYG YLHTFGQGKLEI K	
ALG.AP V-187 ALG.AP V-210 ALG.AP V-223	HCDR1	GGCTTCACATTCAGCAG CTATGCT	29	GFTFSSYA	30
	HCDR2	ATCTCCGGCAGCGGCGG AAGCACC	31	ISGSGGST	32
	HCDR3	GCCAGGTACTATGGCGG CTACTACTCCGCCTGGAT GGACTAC	33	ARYYGGYYSAWM DY	34
	LCDR1	CAGTCCATCTCCAGCTTC	41	QSISSF	42
	LCDR2	GCCGCTTCC	9	AAS	10
	LCDR3	CAGCAGACCTATGGCTA CCTGCACACC	35	QQTYGYLHT	36
	V _H	GAAGTGCAGCTGCTGGA GTCCGGAGGAGGACTGG TGCAGCCTGGCGGAAGC CTGAGGCTGAGCTGCGC TGCCTCCGGCTTCACATT CAGCAGCTATGCTATGA GCTGGGTGAGGCAAGCC CCTGGAAAGTGCCTGGA GTGGGTGTCCGCTATCTC CGGCAGCGGCGGAAGCA CCTACTACGCTGACTCCG TCAAGGGCAGGTTACCC ATCAGCCGGGACAACAG CAAGAACACCCTGTACC TGCAGATGAATAGCCTC AGGGCTGAAGACACCGC TGTGTACTACTGCGCCAG GTACTATGGCGGCTACT	45	EVQLLESGGGLVQ PGGSLRLSCAASG FTFSSYAMSWVRQ APGKCLEWVSAIS GSGGSTYYADSVK GRFTISRDNKNTL YLQMNSLR AEDT AVYYCARYYGGY YSAWMDYWGGQ TLVTVSS	46

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
		ACTCCGCCTGGATGGAC TACTGGGGACAGGGCAC ACTGGTGACCGTGTCCAGC			
	V _L	GATATTCAGATGACACA GTCCCCTAGCTCCCTGTC CGCCAGCGTGGGAGATC GGGTGACCATCACCTGC AGGGCCAGCCAGTCCAT CTCCAGCTTCTTAAACTG GTACCAGCAGAAGCCTG GAAAGGCTCCCAAGCTG CTGATCTACGCCGCTTCC AGCCTCCAGAGCGGCGT GCCTAGCAGGTTCTCCG GCTCCGGAAGCGGAACA GACTTCACCCTGACCATC AGCTCCCTGCAGCCCGA GGACTCCGCTACCTACT ACTGCCAGCAGACCTAT GGCTACCTGCACACCTTC GGCTGCGGCACAAAGCT GGAGATCAAG	47	DIQMTQSPSSLSAS VGDRVITICRASQ SISSFLNWFYQQK GKAPKLLIYAASS LQSGVPSRFSGSGS GTDFTLTISLQPE DSATYYCQQTYG YLHTFGCGTKLEIK	48
ALG.AP V-191	HCDR1	GGCTTCACATTCAGCAGCTATGCT	29	GFTFSSYA	30
	HCDR2	ATCTCCGGCAGCGGCGGAAGCACC	31	ISGSGGST	32
	HCDR3	GCCAGGTACTATGGCGGCTACTACTCCGCCTGGATGGACTAC	33	ARYYGGYYSAWMDY	34
	LCDR1	CAGTCCATCTCCAGCTTC	41	QSISSF	42
	LCDR2	GCCGCTTCC	9	AAS	10
	LCDR3	CAGCAGACCTATGGCTACCTGCACACC	35	QQTYGYLHT	36
	V _H	GAAGTGCAGCTGCTGGA GTCCGGAGGAGGACTGG	45	EVQLLESGGGLVQPGGSLRLSCAASG	46

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
ALG.AP V-196 ALG.AP V-198		TGCAGCCTGGCGGAAGC CTGAGGCTGAGCTGCGC TGCCTCCGGCTTCACATT CAGCAGCTATGCTATGA GCTGGGTGAGGCAAGCC CCTGGAAAGTGCCTGGA GTGGGTGTCCGCTATCTC CGGCAGCGGCGGAAGCA CCTACTACGCTGACTCCG TCAAGGGCAGGTTACCC ATCAGCCGGGACAACAG CAAGAACACCCTGTACC TGCAGATGAATAGCCTC AGGGCTGAAGACACCGC TGTGTACTACTGCGCCAG GTACTATGGCGGCTACT ACTCCGCCTGGATGGAC TACTGGGGACAGGGCAC ACTGGTGACCGTGTCCA GC		FTFSSYAMSWVRQ APGKCLEWVSAIS GSGGSTYYADSVK GRFTISRDNKNTL YLQMNSLRAEDT AVYYCARYYGGY YSAWMDYWGQG TLVTVSS	
	V _L	GATATTCAGATGACACA GTCCCCTAGCTCCCTGTC CGCCAGCGTGGGAGATC GGGTGACCATCACCTGC AGGGCCAGCCAGTCCAT CTCCAGCTTCTTAAACTG GTACCAGCAGAAGCCTG GAAAGGCTCCCAAGCTG CTGATCTACGCCGCTTCC AGCCTCCAGAGCGGCGT GCCTAGCAGGTTCTCCG GCTCCGGAAGCGGAACA GACTTCACCCTGACCATC AGCTCCCTGCAGCCCGA GGACTTCGCTACCTACTA CTGCCAGCAGACCTATG GCTACCTGCACACCTTCG GCTGCGGCACAAAGCTG GAGATCAAG	49	DIQMTQSPSSLSAS VGDRVITITCRASQ SISSFLNWFYQQKP GKAPKLLIYAASS LQSGVPSRFSGSGS GTDFTLTISLQPE DFATYYCQQT YLHTFGCGTKLEIK	50
	HCDR1	GGCTTCGACTTCGAGAG CTATGCT	51	GFDFFESYA	52
	HCDR2	ATCTCCGGCAGCGGCGG AAGCACC	31	ISGSGGST	32

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
	HCDR3	GCCAGGTACTATGGCGGCTACTACTCCGCCTGGATGGACTAC	33	ARYYGGYYSAWMDY	34
	LCDR1	CAGTCCATCAGGAGCGCC	53	QSIRSA	54
	LCDR2	GCCGCTTCC	9	AAS	10
	LCDR3	CAGCAGACCTATGGCTACCTGCACACC	35	QQTYGYLHT	36
	V _H	GAAGTGCAGCTGCTGGA GTCCGGAGGAGGACTGG TGCAGCCTGGCGGAAGC CTGAGGCTGAGCTGCGC TGCCTCCGGCTTCGACTT CGAGAGCTATGCTATGA GCTGGGTGAGGCAAGCC CCTGGAAAGTGCCTGGA GTGGGTGTCCGCTATCTC CGGCAGCGGCGGAAGCA CCTACTACGCTGACTCCG TCAAGGGCAGGTTACCC ATCAGCCGGGACAACAG CAAGAACACCCTGTACC TGCAGATGAATAGCCTC AGGGCTGAAGACACCGC TGTGTACTACTGCGCCAG GTACTATGGCGGCTACT ACTCCGCCTGGATGGAC TACTGGGGACAGGGCAC ACTGGTGACCGTGTCCAGC	55	EVQLLESGGGLVQ PGGSLRLSCAASG FDFESYAMSWVR QAPGKCLEWVSAI SGSGGSTYYADSV KGRFTISRDN SKN TLYLQMNSLRAED TAVYYCARYYGG YYSAWMDYWGQ GTLVTVSS	56
	V _L	GATATTCAGATGACACAGTCCCCTAGCTCCCTGTC CGCCAGCGTGGGAGATCGGGTGACCATCACCTGC AGGGCCAGCCAGTCCATCAGGAGCGCCCTGAACT GGTACCAGCAGAAGCCTGGAAAGGCTCCCAAGCT GCTGATCTACGCCGCTTCAGCCTCCAGAGCGGCG TGCCTAGCAGGTTCTCCGGCTCCGGAAGCGGAACA GACTTCACCCTGACCATC	57	DIQMTQSPSSLSAS VGDRVITICRASQ SIRSLN WYQQKPK GKAPKLLIYAASS LQSGVPSRFSGSGS GTDFTLTISSLQPE DFATYYCQQTYG YLHTFGCGTKLEIK	58

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
		AGCTCCCTGCAGCCCGA GGACTTCGCTACCTACTA CTGCCAGCAGACCTATG GCTACCTGCACACCTTCG GCTGCGGCACAAAGCTG GAGATCAAG			
ALG.AP V-199	HCDR1	GGCTTCGACTTCGACAG CTATGCT	59	GFDFDSYA	60
	HCDR2	ATCTCCGGCAGGGGCGG AAGCACC	61	ISGRGGST	62
	HCDR3	GCCAGGTACTATGGCGG CTACTACTCCGCCTGGAT GGACTAC	33	ARYYGGYYSAWM DY	34
	LCDR1	CAGTCCATCAGGAGCGC C	53	QSIRSA	54
	LCDR2	GCCGCTTCC	9	AAS	10
	LCDR3	CAGCAGACCTATGGCTA CCTGCACACC	35	QQTYGYLHT	36
	V _H	GAAGTGCAGCTGCTGGA GTCCGGAGGAGGACTGG TGCAGCCTGGCGGAAGC CTGAGGCTGAGCTGCGC TGCCTCCGGCTTCGACTT CGACAGCTATGCTATGA GCTGGGTGAGGCAAGCC CCTGGAAAGTGCCTGGA GTGGGTGTCCGCTATCTC CGGCAGGGGCGGAAGCA CCTACTACGCTGACTCCG TCAAGGGCAGGTTACCC ATCAGCCGGGACAACAG CAAGAACACCCTGTACC TGCAGATGAATAGCCTC AGGGCTGAAGACACCGC TGTGTACTACTGCGCCAG GTACTATGGCGGCTACT ACTCCGCCTGGATGGAC TACTGGGGACAGGGCAC ACTGGTGACCGTGTCCA GC	63	EVQLLES GGGLVQ PGGSLRLS CAASG FDFDSYAMSWVR QAPGKCLEWVSAI SGRGGSTYYADSV KGRFTISRDN SKN TLYLQMNSLRAED TAVYYCARYYGG YYSAWMDYWGQ GTLVTVSS	64

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
	V _L	GATATTCAGATGACACA GTCCCCTAGCTCCCTGTC CGCCAGCGTGGGAGATC GGGTGACCATCACCTGC AGGGCCAGCCAGTCCAT CAGGAGCGCCCTGAACT GGTACCAGCAGAAGCCT GGAAAGGCTCCCAAGCT GCTGATCTACGCCGCTTC CAGCCTCCAGAGCGGCG TGCCTAGCAGGTTCTCCG GCTCCGGAAGCGGAACA GACTTCACCCTGACCATC AGCTCCCTGCAGCCCGA GGACTTCGCTACCTACTA CTGCCAGCAGACCTATG GCTACCTGCACACCTTCG GCTGCGGCACAAAGCTG GAGATCAAG	57	DIQMTQSPSSLSAS VGDRVITITCRASQ SIRSALNWYQQKP GKAPKLLIYAASS LQSGVPSRFSGSGS GTDFTLTISLQPE DFATYYCQQTYG YLHTFGCGTKLEI K	58
ALG.AP V-004	HCDR1	GGATTCACCTTTAGCAGC TATGCC	29	GFTFSSYA	30
	HCDR2	ATTAGTGGTAGTGGTGG TAGCACA	31	ISGSGGST	32
	HCDR3	GCGCGCTACTACGGTGG TTACTACTCTGCTTGGAT GGACTAT	33	ARYYGGYYSAWM DY	34
	LCDR1	CAGAGCATTAGCAGCTA T	7	QSISSY	8
	LCDR2	GCTGCATCC	9	AAS	10
	LCDR3	CAACAGACTTACGGTTA CCTGCACACT	35	QQTYGYLHT	36
	V _H	GAGGTGCAGCTGCTCGA GAGCGGGGGAGGCTTGG TACAGCCTGGGGGGTCC CTGCGCCTCTCCTGTGCA GCCAGCGGATTCACCTTT AGCAGCTATGCCATGAG CTGGGTCCGCCAGGCTC CAGGGAAGTGTCTGGAG TGGGTCTCAGCTATTAGT GGTAGTGGTGGTAGCAC ATACTATGCAGACTCCGT GAAGGGCCGGTTCACCA	45	EVQLLESGGGLVQ PGGSLRLSCAASG FTFSSYAMSWVRQ APGKCLEWVSAIS GSGGSTYYADSVK GRFTISRDN SKNTL YLQMNSLR AEDT AVYYCARYYGGY YSAWMDYW GQG TLVTVSS	46

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
		TCTCCCGTGACAATTCCA AGAACACGCTGTATCTG CAAATGAACAGCCTGCG TGCCGAGGACACGGCTG TATATTATTGTGCGCGCT ACTACGGTGGTTACTACT CTGCTTGGATGGACTATT GGGGCCAGGGAACCCTG GTCACCGTCTCCTCA			
	V _L	GACATCCAGATGACCCA GTCTCCATCCTCCCTGAG CGCATCTGTAGGAGACC GCGTCACCATCACTTGCC GGGCAAGTCAGAGCATT AGCAGCTATTTAAATTG GTATCAGCAGAAACCAG GGAAAGCCCCTAAGCTC CTGATCTATGCTGCATCC AGTTTGCAAAGTGGGGT CCCATCACGTTTCAGTGG CAGTGGAAGCGGGACAG ATTCACTCTCACCATCA GCAGTCTGCAACCTGAA GATTTTGCAACTTATTAC TGTC AACAGACTTACGG TTACCTGCACACTTTTGG CTGTGGGACCAGGCTGG AGATCAAA	65	DIQMTQSPSSLSAS VGDRVITICRASQ SISSYLNWYQQKP GKAPKLLIYAASS LQSGVPSRFSGSGS GTDFTLTISLQPE DFATYYCQQTYG YLHTFGCGTRLEI K	66
ALG.AP V-006 ALG.AP V-010	HCDR1	GGATTCACCTTTAGCAGC TATGCC	29	GFTFSSYA	30
	HCDR2	ATTAGTGGTAGTGGTGG TAGACA	31	ISGSGGST	32
	HCDR3	GCGCGCTACTACGGTGG TTACTACTCTGCTTGGAT GGACTAT	33	ARYYGGYYSAWM DY	34
	LCDR1	CAGAGCATTAGCAGCTA T	7	QSISSY	8
	LCDR2	GCTGCATCC	9	AAS	10
	LCDR3	CAACAGACTTACGGTTA CCTGCACACT	35	QQTYGYLHT	36

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
	V _H	GAGGTGCAGCTGTTGGA GAGCGGGGGAGGCTTGG TACAGCCTGGGGGGTCC CTGCGCCTCTCCTGTGCA GCCAGCGGATTCACCTTT AGCAGCTATGCCATGAG CTGGGTCCGCCAGGCTC CAGGGAAGGGGCTGGAG TGGGTCTCAGCTATTAGT GGTAGTGGTGGTAGCAC ATACTATGCAGACTCCGT GAAGGGCCGGTTCACCA TCTCCCGTGACAATTCCA AGAACACGCTGTATCTG CAAATGAACAGCCTGCG TGCCGAGGACACGGCTG TATATTATTGTGCGCGCT ACTACGGTGGTTACTACT CTGCTTGGATGGACTATT GGGGCCAGGGAACCCTG GTCACCGTCTCCTCA	37	EVQLLESGGGLVQ PGGSLRLSCAASG FTFSSYAMSWVRQ APGKGLEWVSAIS GSGGSTYYADSVK GRFTISRDNSKNTL YLQMNSLRAEDT AVYYCARYYGGY YSAWMDYWGQG TLVTVSS	38
	V _L	GACATCCAGATGACCCA GTCTCCATCCTCCCTGAG CGCATCTGTAGGAGACC GCGTCACCATCACTTGCC GGGCAAGTCAGAGCATT AGCAGCTATTTAAATTG GTATCAGCAGAAACCAG GGAAAGCCCCTAAGCTC CTGATCTATGCTGCATCC AGTTTGCAAAGTGGGGT CCCATCACGTTTCAGTGG CAGTGGAAGCGGGACAG ATTTCACTCTCACCATCA GCAGTCTGCAACCTGAA GATTTTGCAACTTATTAC TGTCAACAGACTTACGG TTACCTGCACACTTTTGG CCAGGGGACCAAGCTGG AGATCAAA	67	DIQMTQSPSSLSAS VGDRVITITCRASQ SISSYLNWYQQKP GKAPKLLIYAASS LQSGVPSRFSGSGS GTDFTLTISLQPE DFATYYCQQTYG YLHTFGQGGTKLEI K	68
ALG.AP V-014	HCDR1	GGATTCACCTTTAGCAGC TATGCC	29	GFTFSSYA	30

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
ALG.AP V-018	HCDR2	ATTAGTGGTAGTGGTGG TAGCACA	31	ISGSGGST	32
	HCDR3	GCGCGCTACTACGGTGG TTACTACTCTGCTTGGAT GGACTAT	33	ARYYGGYYSAWM DY	34
	LCDR1	CAGAGCATTAGCAGCTA T	7	QSISSY	8
	LCDR2	GCTGCATCC	9	AAS	10
	LCDR3	CAACAGACTTACGGTTA CCTGCACACT	35	QQTYGYLHT	36
	V _H	GAGGTGCAGCTGTTGGA GAGCGGGGGAGGCTTGG TACAGCCTGGGGGGTCC CTGCGCCTCTCCTGTGCA GCCAGCGGATTCACCTT AGCAGCTATGCCATGAG CTGGGTCCGCCAGGCTC CAGGGAAGTGCCTGGAG TGGGTCTCAGCTATTAGT GGTAGTGGTGGTAGCAC ATACTATGCAGACTCCGT GAAGGGCCGGTTCACCA TCTCCCGTGACAATTCCA AGAACACGCTGTATCTG CAAATGAACAGCCTGCG TGCCGAGGACACGGCTG TATATTATTGTGCGCGCT ACTACGGTGGTTACTACT CTGCTTGGATGGACTATT GGGGCCAGGGAACCCTG GTCACCGTCTCCTCA	45	EVQLLESGGGLVQ PGGSLRLSCAASG FTFSSYAMSWVRQ APGKCLEWVSAIS GSGGSTYYADSVK GRFTISRDN SKNTL YLQMNSLRAEDT AVYYCARYYGGY YSAWMDYWGQG TLVTVSS	46
	V _L	GACATCCAGATGACCCA GTCTCCATCCTCCCTGAG CGCATCTGTAGGAGACC GCGTCACCATCACTTGCC GGGCAAGTCAGAGCATT AGCAGCTATTTAAATTG GTATCAGCAGAAACCAG GGAAAGCCCCTAAGCTC CTGATCTATGCTGCATCC AGTTTGCAAAGTGGGGT CCCATCACGTTTCAGTGG CAGTGGAAGCGGGACAG	69	DIQMTQSPSSLSAS VGDRVITICRASQ SISSYLNWYQQKP GKAPKLLIYAASS LQSGVPSRFSGSGS GTDFTLTISLQPE DFATYYCQQTYG YLHTFGCGTKLEI K	70

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
		ATTTCACTCTCACCATCA GCAGTCTGCAACCTGAA GATTTTGCAACTTATTAC TGTCAACAGACTTACGG TTACCTGCACACTTTTGG CTGCGGGACCAAGCTGG AGATCAAA			

[00192] In some embodiments, the present invention provides for multispecific polypeptides (*e.g.*, bispecific polypeptides) comprising a 5T4-binding domain. In such embodiments, a 5T4-binding protein or polypeptide can comprise one or more additional binding domains (*e.g.*, second binding domain) that bind a target other than 5T4. These other binding domains can comprise, for example, a particular cytokine or a molecule that targets the binding domain polypeptide to, for example, a particular cell type, a toxin, an additional cell receptor, or an antibody. A multispecific 5T4-binding polypeptide or protein may comprise two binding domains (the domains can be designed to specifically bind the same or different targets), a hinge region, a linker (*e.g.*, a carboxyl-terminus or an amino-terminus linker), and an immunoglobulin constant region. A multispecific 5T4-binding protein may be a homodimeric protein comprising two identical, disulfide-bonded polypeptides. In some embodiments the 5T4-binding domains may be derived from a monoclonal antibody that binds to 5T4.

[00193] In some embodiments of the disclosure, a 5T4-binding polypeptide is capable of forming a heterodimer with a second polypeptide chain and comprises a hinge region (a) immediately amino-terminal to an immunoglobulin constant region (*e.g.*, amino-terminal to a CH2 domain wherein the immunoglobulin constant region includes CH2 and CH3 domains, or amino-terminal to a CH3 domain wherein the immunoglobulin sub-regions includes CH3 and CH4 domains), (b) interposed between and connecting a binding domain (*e.g.*, scFv) and a immunoglobulin heterodimerization domain, (c) interposed between and connecting a immunoglobulin heterodimerization domain and an immunoglobulin constant region (*e.g.*, wherein the immunoglobulin constant region includes CH2 and CH3 domains or CH3 and CH4 domains), (d) interposed between and connecting an immunoglobulin constant region and a binding domain, (e) at the amino-terminus of a polypeptide chain, or (f) at the carboxyl-terminus

of a polypeptide chain. A polypeptide chain comprising a hinge region as described herein will be capable of associating with a different polypeptide chain to form a heterodimeric protein provided herein, and the heterodimer formed will contain a binding domain that retains its target specificity or its specific target binding affinity.

[00194] In some embodiments, the 5T4-binding polypeptide provided herein is a polypeptide comprising two scFvs. In some embodiments, the two scFvs comprise 5T4-binding domains. In certain embodiments, the two scFvs comprise identical 5T4-binding domains. In other embodiments, the two scFvs comprise different 5T4-binding domains. In other embodiments, the polypeptide comprises an 5T4-binding domain as a first scFv and an effector cell binding domain as a second scFv. For example, the effector cell binding domain may be an scFv specific for 4-1BB.

[00195] In some embodiments, a 5T4-binding polypeptide provided herein comprises an anti-5T4 scFv that is at least about 82%, at least about 85%, at least about 87%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 118, 120, 122, 124, 126, 128, 130, 132, 134, and 170, wherein the polypeptide is a multispecific polypeptide in the format scFv-Fc-scFv and comprises a Y to F substitution in the LCDR1 at position 99 of the anti-5T4 V_L and/or a F to S substitution in the FR3 at position 148 of the anti-5T4 V_L. In some embodiments, a 5T4-binding polypeptide provided herein comprises an anti-5T4 scFv that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 118, 120, 122, 124, 126, 128, 130, 132, 134, and 170. Amino acid and nucleic acid sequences for exemplary anti-5T4 scFvs are provided in Table 6 below.

Table 6. Amino Acid and DNA Sequences of Exemplary anti-5T4 scFvs

Name	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
ALG.AP V-178	GAAGTGCAGCTGCTGGAGTCCGG	117	EVQLLESGGGLV	118
	AGGAGGACTGGTGCAGCCTGGC		QPGGSLRLSCAAS	
ALG.AP V-208	GGAAGCCTGAGGCTGAGCTGCGC		GFTFSSYAMSWV	
	TGCCTCCGGCTTCACATTCAGCA		RQAPGKGLEWVS	
anti-5T4	GCTATGCTATGAGCTGGGTGAGG		AISGSGGSTYYAD	
	CAAGCCCCTGGAAAGGGCCTGG		SVKGRFTISRDN	

Name	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
	AGTGGGTGTCCGCTATCTCCGGC AGCGGCGGAAGCACCTACTACGC TGACTCCGTCAAGGGCAGGTTCA CCATCAGCCGGGACAACAGCAA GAACACCCTGTACCTGCAGATGA ATAGCCTCAGGGCTGAAGACACC GCTGTGTACTACTGCGCCAGGTA CTATGGCGGCTACTACTCCGCCT GGATGGACTACTGGGGACAGGG CACACTGGTGACCGTGTCCAGCG GCGGAGGCGGCTCCGGAGGCGG TGGCTCCGGAGGAGGCGGAAGC GGAGGAGGAGGCTCCGATATTCA GATGACACAGTCCCCTAGCTCCC TGTCCGCCAGCGTGGGAGATCGG GTGACCATCACCTGCAGGGCCAG CCAGTCCATCTCCAGCTATTTAA ACTGGTACCAGCAGAAGCCTGGA AAGGCTCCCAAGCTGCTGATCTA CGCCGCTTCCAGCCTCCAGAGCG GCGTGCCTAGCAGGTTCTCCGGC TCCGGAAGCGGAACAGACTTCAC CCTGACCATCAGCTCCCTGCAGC CCGAGGACTCCGCTACCTACTAC TGCCAGCAGACCTATGGCTACCT GCACACCTTCGGCCAGGGCACAA AGCTGGAGATCAAG		KNTLYLQMNSLR AEDTAVYYCARY YGGYYSAWMDY WGQGTTLTVSSG GGGSGGGGSGGG GSGGGGSDIQMT QSPSSLSASVGDR VTITCRASQSISSY LNWYQQKPGKAP KLLIYAASSLQSG VPSRFSGSGSGTD FTLTISLQPEDSA TYYCQQTYGYLH TFGQGTKLEIK	
ALG.AP V-179 ALG.AP V.209 ALG.AP V222 anti-5T4	GAAGTGCAGCTGCTGGAGTCCGG AGGAGGACTGGTGCAGCCTGGC GGAAGCCTGAGGCTGAGCTGCGC TGCCTCCGGCTTCACATTCAGCA GCTATGCTATGAGCTGGGTGAGG CAAGCCCCTGGAAAGGGCCTGG AGTGGGTGTCCGCTATCTCCGGC AGCGGCGGAAGCACCTACTACGC TGACTCCGTCAAGGGCAGGTTCA CCATCAGCCGGGACAACAGCAA GAACACCCTGTACCTGCAGATGA ATAGCCTCAGGGCTGAAGACACC GCTGTGTACTACTGCGCCAGGTA CTATGGCGGCTACTACTCCGCCT GGATGGACTACTGGGGACAGGG CACACTGGTGACCGTGTCCAGCG	119	EVQLLESGGGLV QPGGSLRLSCAAS GFTFSSYAMSWV RQAPGKGLEWVS AISGSGGSTYYAD SVKGRFTISRDN KNTLYLQMNSLR AEDTAVYYCARY YGGYYSAWMDY WGQGTTLTVSSG GGGSGGGGSGGG GSGGGGSDIQMT QSPSSLSASVGDR VTITCRASQSISSF LNWYQQKPGKAP KLLIYAASSLQSG	120

Name	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
	GCGGAGGCGGCTCCGGAGGCGG TGGCTCCGGAGGAGGCGGAAGC GGAGGAGGAGGCTCCGATATTCA GATGACACAGTCCCCTAGCTCCC TGTCCGCCAGCGTGGGAGATCGG GTGACCATCACCTGCAGGGCCAG CCAGTCCATCTCCAGCTTCTTAA ACTGGTACCAGCAGAAGCCTGGA AAGGCTCCCAAGCTGCTGATCTA CGCCGCTTCCAGCCTCCAGAGCG GCGTGCCTAGCAGGTTCTCCGGC TCCGGAAGCGGAACAGACTTCAC CCTGACCATCAGCTCCCTGCAGC CCGAGGACTCCGCTACCTACTAC TGCCAGCAGACCTATGGCTACCT GCACACCTTCGGCCAGGGCACAA AGCTGGAGATCAAG		VPSRFSGSGSGTD FTLTISSLQPEDSA TYYCQQTYGYLH TFGQGTKLEIK	
ALG.AP V-187 ALG.AP V.210 ALG.AP V223 anti-5T4	GAAGTGCAGCTGCTGGAGTCCGG AGGAGGACTGGTGCAGCCTGGC GGAAGCCTGAGGCTGAGCTGCGC TGCCTCCGGCTTCACATTCAGCA GCTATGCTATGAGCTGGGTGAGG CAAGCCCCTGGAAAGTGCCTGGA GTGGGTGTCCGCTATCTCCGGCA GCGGCGGAAGCACCTACTACGCT GACTCCGTCAAGGGCAGGTTCAC CATCAGCCGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAA TAGCCTCAGGGCTGAAGACACCG CTGTGTACTACTGCGCCAGGTAC TATGGCGGCTACTACTCCGCCTG GATGGACTACTGGGGACAGGGC ACACTGGTGACCGTGTCCAGCGG CGGAGGCGGCTCCGGAGGCGGT GGCTCCGGAGGAGGCGGAAGCG GAGGAGGAGGCTCCGATATTAG ATGACACAGTCCCCTAGCTCCCT GTCCGCCAGCGTGGGAGATCGGG TGACCATCACCTGCAGGGCCAGC CAGTCCATCTCCAGCTTCTTAAA CTGGTACCAGCAGAAGCCTGGAA AGGCTCCCAAGCTGCTGATCTAC GCCGCTTCCAGCCTCCAGAGCGG	121	EVQLLESGGGLV QPGGSLRLSCAAS GFTFSSYAMSWV RQAPGKCLEWVS AISGSGGSTYYAD SVKGRFTISRDN KNTLYLQMNSLR AEDTAVYYCARY YGGYSAWMDY WGQGTLVTVSSG GGGSGGGSGGG GSGGGGSDIQMT QSPSSLSASVGDR VTITCRASQSISSF LNWYQQKPGKAP KLLIYAASSLQSG VPSRFSGSGSGTD FTLTISSLQPEDSA TYYCQQTYGYLH TFGCGTKLEIK	122

Name	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
	CGTGCCTAGCAGGTTCTCCGGCT CCGGAAGCGGAACAGACTTCACC CTGACCATCAGCTCCCTGCAGCC CGAGGACTCCGCTACCTACTACT GCCAGCAGACCTATGGCTACCTG CACACCTTCGGCTGCGGCACAAA GCTGGAGATCAAG			
ALG.AP V-191 anti-5T4	GAAGTGCAGCTGCTGGAGTCCGG AGGAGGACTGGTGCAGCCTGGC GGAAGCCTGAGGCTGAGCTGCGC TGCCTCCGGCTTCACATTCAGCA GCTATGCTATGAGCTGGGTGAGG CAAGCCCCTGGAAAGTGCCTGGA GTGGGTGTCCGCTATCTCCGGCA GCGGCGGAAGCACCTACTACGCT GACTCCGTCAAGGGCAGGTTAC CATCAGCCGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAA TAGCCTCAGGGCTGAAGACACCG CTGTGTACTACTGCGCCAGGTAC TATGGCGGCTACTACTCCGCCTG GATGGACTACTGGGGACAGGGC ACACTGGTGACCGTGTCCAGCGG CGGAGGCGGCTCCGGAGGCGGT GGCTCCGGAGGAGGCGGAAGCG GAGGAGGAGGCTCCGATATTCAG ATGACACAGTCCCCTAGCTCCCT GTCCGCCAGCGTGGGAGATCGGG TGACCATCACCTGCAGGGCCAGC CAGTCCATCTCCAGCTTCTTAAA CTGGTACCAGCAGAAGCCTGGAA AGGCTCCCAAGCTGCTGATCTAC GCCGCTTCCAGCCTCCAGAGCGG CGTGCCTAGCAGGTTCTCCGGCT CCGGAAGCGGAACAGACTTCACC CTGACCATCAGCTCCCTGCAGCC CGAGGACTTCGCTACCTACTACT GCCAGCAGACCTATGGCTACCTG CACACCTTCGGCTGCGGCACAAA GCTGGAGATCAAG	123	EVQLLESGGGLV QPGGSLRLSCAAS GFTFSSYAMSWV RQAPGKCLEWVS AISGSGGSTYYAD SVKGRFTISRDN KNTLYLQMNSLR AEDTAVYYCARY YGGYSAWMDY WGQGTLLTVSSG GGGSGGGSGGG GSGGGGSDIQMT QSPSSLSASVGDR VTITCRASQSISS LNWYQQKPGKAP KLLIYAASSLQSG VPSRFGSGSGTD FTLTISLQPEDFA TYYCQQTYGYLH TFGCGTKLEIK	124
ALG.AP V-196 anti-5T4	GAAGTGCAGCTGCTGGAGTCCGG AGGAGGACTGGTGCAGCCTGGC GGAAGCCTGAGGCTGAGCTGCGC	125	EVQLLESGGGLV QPGGSLRLSCAAS GFDFESYAMSWV	126

Name	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
ALG.AP V-198 anti-5T4	TGCCTCCGGCTTCGACTTCGAGA GCTATGCTATGAGCTGGGTGAGG CAAGCCCCTGGAAAGTGCCTGGA GTGGGTGTCCGCTATCTCCGGCA GCGGCGGAAGCACCTACTACGCT GACTCCGTCAAGGGCAGGTTCAC CATCAGCCGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAA TAGCCTCAGGGCTGAAGACACCG CTGTGTACTACTGCGCCAGGTAC TATGGCGGCTACTACTCCGCCTG GATGGACTACTGGGGACAGGGC ACACTGGTGACCGTGTCCAGCGG CGGAGGCGGCTCCGGAGGCGGT GGCTCCGGAGGAGGCGGAAGCG GAGGAGGAGGCTCCGATATTAG ATGACACAGTCCCCTAGCTCCCT GTCCGCCAGCGTGGGAGATCGGG TGACCATCACCTGCAGGGCCAGC CAGTCCATCAGGAGCGCCCTGAA CTGGTACCAGCAGAAGCCTGGAA AGGCTCCCAAGCTGCTGATCTAC GCCGCTTCCAGCCTCCAGAGCGG CGTGCCTAGCAGGTTCTCCGGCT CCGGAAGCGGAACAGACTTCACC CTGACCATCAGCTCCCTGCAGCC CGAGGACTTCGCTACCTACTACT GCCAGCAGACCTATGGCTACCTG CACACCTTCGGCTGCGGCACAAA GCTGGAGATCAAG		RQAPGKCLEWVS AISGSGGSTYYAD SVKGRFTISRDN KNTLYLQMNSLR AEDTAVYYCARY YGGYSAWMDY WGQGLVTVSSG GGGSGGGSGGG GSGGGGSDIQMT QSPSSLSASVGDR VTITCRASQSIRSA LNWYQQKPGKAP KLLIYAASSLQSG VPSRFSGSGSGTD FTLTISSLQPEDFA TYYCQQTYGYLH TFGCGTKLEIK	
ALG.AP V-199 anti-5T4	GAAGTGCAGCTGCTGGAGTCCGG AGGAGGACTGGTGCAGCCTGGC GGAAGCCTGAGGCTGAGCTGCGC TGCCTCCGGCTTCGACTTCGACA GCTATGCTATGAGCTGGGTGAGG CAAGCCCCTGGAAAGTGCCTGGA GTGGGTGTCCGCTATCTCCGGCA GGGGCGGAAGCACCTACTACGCT GACTCCGTCAAGGGCAGGTTCAC CATCAGCCGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAA TAGCCTCAGGGCTGAAGACACCG CTGTGTACTACTGCGCCAGGTAC	127	EVQLLESGGGLV QPGGSLRLSCAAS GFDYFDSYAMSWV RQAPGKCLEWVS AISGRGGSTYYA DSVKGRFTISRDN SKNTLYLQMNSL RAEDTAVYYCAR YGGYSAWMD YWGQGLVTVSS GGGGSGGGSGG GGSGGGGSDIQM TQSPSSLSASVGD	128

Name	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
	TATGGCGGCTACTACTCCGCCTG GATGGACTACTGGGGACAGGGC ACACTGGTGACCGTGTCCAGCGG CGGAGGCGGCTCCGGAGGCGGT GGCTCCGGAGGAGGCGGAAGCG GAGGAGGAGGCTCCGATATTCAG ATGACACAGTCCCCTAGCTCCCT GTCCGCCAGCGTGGGAGATCGGG TGACCATCACCTGCAGGGCCAGC CAGTCCATCAGGAGCGCCCTGAA CTGGTACCAGCAGAAGCCTGGAA AGGCTCCCAAGCTGCTGATCTAC GCCGCTTCCAGCCTCCAGAGCGG CGTGCCTAGCAGGTTCTCCGGCT CCGGAAGCGGAACAGACTTCACC CTGACCATCAGCTCCCTGCAGCC CGAGGACTTCGCTACCTACTACT GCCAGCAGACCTATGGCTACCTG CACACCTTCGGCTGCGGCACAAA GCTGGAGATCAAG		RVTITCRASQSIRS ALNWFYQQKPGK APKLLIYAASSLQ SGVPSRFSGSGSG TDFTLTISSLQPED FATYYCQQTYGY LHTFGCGTKLEIK	
ALG.AP V-006 anti-5T4	GAGGTGCAGCTGTTGGAGAGCG GGGGAGGCTTGGTACAGCCTGGG GGGTCCCTGCGCCTCTCCTGTGC AGCCAGCGGATTACCTTTAGCA GCTATGCCATGAGCTGGGTCCGC CAGGCTCCAGGGAAGGGGCTGG AGTGGGTCTCAGCTATTAGTGGT AGTGGTGGTAGCACATACTATGC AGACTCCGTGAAGGGCCGGTTCA CCATCTCCCGTGACAATTCCAAG AACACGCTGTATCTGCAAATGAA CAGCCTGCGTGCCGAGGACACGG CTGTATATTATTGTGCGCGCTACT ACGGTGGTTACTACTCTGCTTGG ATGGACTATTGGGGCCAGGGAAC CCTGGTCACCGTCTCCTCAGGCG GTGGAGGCAGCGGTGGGGGTGG GTCTGGAGGCGGTGGCAGTGGCG GCGGAGGCTCTGACATCCAGATG ACCCAGTCTCCATCCTCCCTGAG CGCATCTGTAGGAGACCGCGTCA CCATCACTTGCCGGGCAAGTCAG AGCATTAGCAGCTATTAAATTG	129	EVQLLESGGGLV QPGGSLRLSCAAS GFTFSSYAMSWV RQAPGKGLEWVS AISGSGGSTYYAD SVKGRFTISRDN KNTLYLQMNSLR AEDTAVYYCARY YGGYSAWMDY WGQGTLVTVSSG GGGSGGGGSGGG GSGGGGSDIQMT QSPSSLSASVGDR VTITCRASQSISSY LNWFYQQKPGKAP KLLIYAASSLQSG VPSRFSGSGSGTD FTLTISSLQPEDFA TYYCQQTYGYLH TFGQGTKLEIK	130

Name	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
	GTATCAGCAGAAACCAGGGAAA GCCCCTAAGCTCCTGATCTATGC TGCATCCAGTTTGCAAAGTGGGG TCCCATCACGTTTCAGTGGCAGT GGAAGCGGGACAGATTTCACTCT CACCATCAGCAGTCTGCAACCTG AAGATTTTGCAACTTATTACTGT CAACAGACTTACGGTTACCTGCA CACTTTTGGCCAGGGGACCAAGC TGGAGATCAAA			
ALG.AP V-010 anti-5T4	GACATCCAGATGACCCAGTCTCC ATCCTCCCTGAGCGCATCTGTAG GAGACCGCGTCACCATCACTTGC CGGGCAAGTCAGAGCATTAGCA GCTATTTAAATTGGTATCAGCAG AAACCAGGGAAAGCCCCTAAGC TCCTGATCTATGCTGCATCCAGTT TGCAAAGTGGGGTCCCATCACGT TTCAGTGGCAGTGGAAGCGGGAC AGATTTCACTCTCACCATCAGCA GTCTGCAACCTGAAGATTTTGCA ACTTATTACTGTCAACAGACTTA CGGTTACCTGCACACTTTTGGCC AGGGGACCAAGCTGGAGATCAA AGGCGGTGGAGGCAGCGGTGGG GGTGGGTCTGGAGGCGGTGGCA GTGGCGGCGGAGGCTCTGAGGTG CAGCTGTTGGAGAGCGGGGGAG GCTTGGTACAGCCTGGGGGGTCC CTGCGCCTCTCCTGTGCAGCCAG CGGATTCACCTTTAGCAGCTATG CCATGAGCTGGGTCCGCCAGGCT CCAGGGAAGGGGCTGGAGTGGG TCTCAGCTATTAGTGGTAGTGGT GGTAGCACATACTATGCAGACTC CGTGAAGGGCCGGTTCACCATCT CCCGTGACAATTCCAAGAACACG CTGTATCTGCAAATGAACAGCCT GCGTGCCGAGGACACGGCTGTAT ATTATTGTGCGCGCTACTACGGT GGTACTACTCTGCTTGGATGGA CTATTGGGGCCAGGGAACCCTGG TCACCGTCTCCTCA	169	DIQMTQSPSSL SA SVGDRVITITCRAS QSISSYLNWYQQ KPGKAPKLLIYAA SSLQSGVPSRFSG SGSGTDFTLTISSL QPEDFATYYCQQ TYGYLHTFGQGT KLEIKGGGSGG GGSGGGSGGGG SEVQLLESGGGL VQPGGSLRLSCA ASGFTFSSYAMS WVRQAPGKGLE WVSAISGSGGSTY YADSVKGRFTISR DNSKNTLYLQMN SLRAEDTAVYYC ARYYGGYSAW MDYWGQGT LVTVSS	170

Name	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
ALG.AP V-014 anti-5T4	GAGGTGCAGCTGTTGGAGAGCG GGGGAGGCTTGGTACAGCCTGGG GGGTCCCTGCGCCTCTCCTGTGC AGCCAGCGGATTACCTTTAGCA GCTATGCCATGAGCTGGGTCCGC CAGGCTCCAGGGAAGTGCCTGGA GTGGGTCTCAGCTATTAGTGGTA GTGGTGGTAGCACATACTATGCA GACTCCGTGAAGGGCCGGTTCAC CATCTCCCGTGACAATTCCAAGA ACACGCTGTATCTGCAAATGAAC AGCCTGCGTGCCGAGGACACGGC TGTATATTATTGTGCGCGCTACT ACGGTGGTTACTACTCTGCTTGG ATGGACTATTGGGGCCAGGGAAC CCTGGTCACCGTCTCCTCAGGCG GTGGAGGCAGCGGTGGGGGTGG GTCTGGAGGCGGTGGCAGTGGCG GCGGAGGCTCTGACATCCAGATG ACCCAGTCTCCATCCTCCCTGAG CGCATCTGTAGGAGACCGCGTCA CCATCACTTGCCGGGCAAGTCAG AGCATTAGCAGCTATTTAAATTG GTATCAGCAGAAACCAGGGAAA GCCCCTAAGCTCCTGATCTATGC TGCATCCAGTTTGCAAAGTGGGG TCCCATCACGTTTCAGTGGCAGT GGAAGCGGGACAGATTTCACTCT CACCATCAGCAGTCTGCAACCTG AAGATTTTGCAACTTATTACTGT CAACAGACTTACGGTTACCTGCA CACTTTTGGCTGCGGGACCAAGC TGGAGATCAA	131	EVQLLESGGGLV QPGGSLRLSCAAS GFTFSSYAMSWV RQAPGKCLEWVS AISGSGGSTYYAD SVKGRFTISRDN KNTLYLQMNSLR AEDTAVYYCARY YGGYYSAWMDY WGQGTLVTVSSG GGGSGGGSGGG GSGGGGSDIQMT QSPSSLASVGDR VTITCRASQSISSY LNWYQQKPGKAP KLLIYAASSLQSG VPSRFSGSGSGTD FTLTISLQPEDFA TYYCQQTYGYLH TFGCGTKLEIK	132
ALG.AP V-018 anti-5T4	GACATCCAGATGACCCAGTCTCC ATCCTCCCTGAGCGCATCTGTAG GAGACCGCGTCACCATCACTTGC CGGGCAAGTCAGAGCATTAGCA GCTATTTAAATTGGTATCAGCAG AAACCAGGGAAAGCCCCTAAGC TCCTGATCTATGCTGCATCCAGTT TGCAAAGTGGGGTCCCATCACGT TTCAGTGGCAGTGGAAGCGGGAC AGATTTCACTCTCACCATCAGCA	133	DIQMTQSPSSL SA SVGDRVTITCRAS QSISSYLNWYQQ KPGKAPKLLIYAA SSLQSGVPSRFSG SGSGTDFTLTISL QPEDFATYYCQQ TYGYLHTFGCGT KLEIKGGGGSGG GGSGGGSGGGG	134

Name	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
	GTCTGCAACCTGAAGATTTTGCA ACTTATTACTGTCAACAGACTTA CGGTTACCTGCACACTTTTGGCT GCGGGACCAAGCTGGAGATCAA AGGCGGTGGAGGCAGCGGTGGG GGTGGGTCTGGAGGCGGTGGCA GTGGCGGCGGAGGCTCTGAGGTG CAGCTGTTGGAGAGCGGGGGAG GCTTGGTACAGCCTGGGGGGTCC CTGCGCCTCTCCTGTGCAGCCAG CGGATTCACCTTTAGCAGCTATG CCATGAGCTGGGTCCGCCAGGCT CCAGGGAAGTGCCTGGAGTGGGT CTCAGCTATTAGTGGTAGTGGTG GTAGCACATACTATGCAGACTCC GTGAAGGGCCGGTTCACCATCTC CCGTGACAATTCCAAGAACACGC TGTATCTGCAAATGAACAGCCTG CGTGCCGAGGACACGGCTGTATA TTATTGTGCGCGCTACTACGGTG GTTACTACTCTGCTTGGATGGAC TATTGGGGCCAGGGAACCCTGGT CACCGTCTCCTCA		SEVQLLESGGGL VQPGGSLRLSCA ASGFTFSSYAMS WVRQAPGKCLE WVSAISGSGGSTY YADSVKGRFTISR DNSKNTLYLQMN SLRAEDTAVYYC ARYYGGYYS AW MDYWGQGTLVT VSS	

[00196] In some embodiments, a 5T4-binding polypeptide comprises, in order from amino-terminus to carboxyl-terminus (or in order from carboxyl-terminus to amino-terminus), (i) a 5T4-binding domain, (ii) a hinge region, (iii) an immunoglobulin constant region, (iv) a carboxyl-terminus linker (or an amino-terminus linker), and (v) a second binding domain. In further embodiments, the second binding domain is an scFv specific for 4-1BB. In some embodiments, a 5T4-binding polypeptide comprises, in order from amino-terminus to carboxyl-terminus (or in order from carboxyl-terminus to amino-terminus), (i) second binding domain, (ii) a hinge region, (iii) an immunoglobulin constant region, (iv) a carboxyl-terminus linker (or an amino-terminus linker), and (v) a 5T4-binding domain. In further embodiments, the second binding domain comprises or is a 4-1BB-binding domain. In certain embodiments, a 5T4-binding protein can comprise an effector-cell binding domain for recruitment of effector to target cells expressing 5T4. In certain embodiments, the effector-cell binding domain specifically binds to 4-1BB.

[00197] In some embodiments, the second binding domain of a 5T4-binding polypeptide described herein is a 4-1BB-binding domain and comprises one or more of the 4-1BB-binding sequences (*e.g.*, CDRs or variable regions) disclosed in PCT Application Publication No WO 2016/185016; PCT Application No. PCT/EP2017/059656; Dubrot *et al.*, 2010; Gauttier *et al.*, 2014; Kim *et al.*, 2001; McMillin *et al.*, 2006; Melero *et al.*, 1997; Miller *et al.*, 2002; Sallin *et al.*, 2014; Taraban *et al.*, 2002; Uno *et al.*, 2006; Vinay and Kwon, 2012; Wilcox *et al.*, 2002, each of which is incorporated herein by reference in its entirety.

[00198] In some embodiments, the second binding domain specifically binds 4-1BB and comprises an immunoglobulin light chain variable region (V_L) and an immunoglobulin heavy chain variable region (V_H); wherein the V_L comprises an amino acid sequence that is at least about 93% identical, at least about 95% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 and 22; and wherein the V_H comprises an amino acid sequence that is at least about 93% identical, at least about 95% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 20, 26, and 28, wherein the polypeptide is a multispecific polypeptide in the format scFv-Fc-scFv and comprises a Y to H substitution in the HCDR1 at position 150 of the V_H and/or an R to H substitution in the FR3 at position 127 of the V_H. In some embodiments, the second binding domain specifically binds 4-1BB and comprises a V_L and a V_H; wherein the V_L comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 and 22; and wherein the V_H comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 20, 26, and 28.

[00199] In some embodiments, the second binding domain is a 4-1BB-binding domain polypeptide, wherein the polypeptide is an scFv comprising a sequence that is at least about 82%, at least about 85%, at least about 87%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 110, 112, 114, and 116. Exemplary anti-4-1BB binding domain sequences are shown in Tables 6 and 7 and are discussed further below.

4-1BB-binding domains and proteins comprising the same

[00200] In some embodiments, the present invention provides 4-1BB-binding domains that specifically bind to 4-1BB (*e.g.* human 4-1BB). 4-1BB is also known as CD137 or TNFRSF9 and is a member of the tumor necrosis factor (TNF) receptor family. 4-1BB is expressed on multiple cell types, including activated subsets of T cell (*e.g.*, CD4+ and CD8+ T cells and T regulatory cells (Tregs)), natural killer (NK) cells, dendritic cells, monocytes, mast cells, and eosinophils. 4-1BB activation on CD8+ T cells sustains and/or augments cellular activation, while 4-1BB activation on CD4+ T cells can initiate cell activation and, in some instance, lead to activation-induced cell death. 4-1BB activation on Tregs generally suppresses Treg function. Several studies have demonstrated induction of tumor immunity through the use of antagonists 4-1BB antibodies. Exemplary human 4-1BB nucleotide and amino acid sequences are provided in SEQ ID NOs: 163, 167 and SEQ ID NOs: 164 and 166, respectively.

[00201] In some embodiments, the present invention further provides polypeptides comprising a 4-1BB-binding domain (*e.g.*, 4-1BB-binding polypeptides). In certain variations, the 4-1BB-binding polypeptide comprises a hinge region carboxyl-terminal to the 4-1BB-binding domain, and an immunoglobulin constant region. In further variations, the 4-1BB-binding polypeptide comprises a carboxyl-terminus binding domain linker carboxyl-terminal to the immunoglobulin constant region, and a second binding domain carboxyl-terminal to the carboxyl-terminus linker.

[00202] In yet other variations, a 4-1BB-binding polypeptide comprises a hinge region amino-terminal to the polypeptide comprising the 4-1BB-binding domain, and an immunoglobulin constant amino-terminal to the hinge region.

[00203] In some embodiments, the 4-1BB-binding domains described herein binds an epitope located on the extracellular domain of 4-1BB (*e.g.*, an epitope comprised within SEQ ID NO: 166). In certain aspects, this epitope is a discontinuous and/or conformational epitope.

[00204] A 4-1BB-binding domain polypeptide may specifically bind to human 4-1BB and comprise a heavy chain CDR1 (HCDR1), HCDR2, HCDR3, light chain CDR1 (LCDR1), LCDR2, and LCDR3, wherein the HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 24; the HCDR2 comprises an amino acid sequence of SEQ ID NO: 4; the HCDR3 comprises an amino acid sequence of SEQ ID NO: 6; the LCDR1 comprises an amino acid sequence of SEQ ID NO: 8; the LCDR2 comprises an amino acid

sequence of SEQ ID NO: 10; and the LCDR3 comprises an amino acid sequence of SEQ ID NO: 12, wherein the polypeptide is a multispecific polypeptide in the format scFv-Fc-scFv and comprises a Y to H substitution in the HCDR1 at position 150 of the anti-4-1BB V_H and/or an R to H substitution in the FR3 at position 127 of the anti-4-1BB V_H.

[00205] In particular embodiments, a 4-1BB-binding domain polypeptide may specifically bind to human 4-1BB and comprise an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 2; (b) the HCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 4; (c) the HCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 6; (d) the LCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 8; the LCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 12. In other particular embodiments, a 5T4-binding domain polypeptide specifically binds to human 5T4 and comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 18; (b) the HCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 4; (c) the HCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 6; (d) the LCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 8; (e) the LCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 10 and (f) the LCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 12. In other particular embodiments, a 5T4-binding domain polypeptide specifically binds to human 5T4 and comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 24; (b) the HCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 4; (c) the HCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 6; (d) the LCDR1 comprises an amino acid sequence set forth in SEQ ID NO: 8; (e) the LCDR2 comprises an amino acid sequence set forth in SEQ ID NO: 10; and (f) the LCDR3 comprises an amino acid sequence set forth in SEQ ID NO: 12.

[00206] In certain embodiments, a 4-1BB-binding domain polypeptide comprises an amino acid sequence of a light chain variable region (V_L) selected from the group consisting of SEQ ID NOs: 16 and 22. In certain embodiments, a 4-1BB-binding domain polypeptide comprises an amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an

amino acid of a heavy chain variable region (V_H) selected from the group consisting of SEQ ID NOs: 14, 20, 26, and 28, wherein the polypeptide is a multispecific polypeptide in the format scFv-Fc-scFv and comprises a Y to H substitution in the HCDR1 at position 150 of the anti-4-1BB V_H and/or an R to H substitution in the FR3 at position 127 of the anti-4-1BB V_H. In certain embodiments, a 4-1BB-binding domain polypeptide comprises an amino acid sequence of a heavy chain variable region (V_H) selected from the group consisting of SEQ ID NOs: 14, 20, 26, and 28.

[00207] In certain embodiments, a 4-1BB-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 14 and a V_L region comprising the amino acid sequence of SEQ ID NO: 16. In certain embodiments, a 4-1BB-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 20 and a V_L region comprising the amino acid sequence of SEQ ID NO: 22. In certain embodiments, a 4-1BB-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 26 and a V_L region comprising the amino acid sequence of SEQ ID NO: 16. In certain embodiments, a 4-1BB-binding domain polypeptide comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 28 and a V_L region comprising the amino acid sequence of SEQ ID NO: 16.

[00208] Exemplary anti-4-1BB binding domain sequences are shown in Table 7.

Table 7. Exemplary 4-1BB binding domain polypeptide and nucleic acid sequences

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
ALG.AP V-178 ALG.AP V-179 ALG.AP V-187 ALG.AP V-198 ALG.AP V-199	HCDR1	GGATTCACCTTTTCTCAC GGTTCT	1	GFTFSHGS	2
	HCDR2	ATTTCTTCTGGTTCTGGTT CTACA	3	ISSGSGST	4
	HCDR3	GCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTG ACTAT	5	ARSSYYGSYY SIDY	6
	LCDR1	CAGAGCATTAGCAGCTAT	7	QSISSY	8
	LCDR2	GCTGCATCC	9	AAS	10
	LCDR3	CAACAGTACTACGACAAC CTGCCCACT	11	QQYYDNLPT	12

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
ALG.AP V-208 ALG.AP V-209 ALG.AP V-210 ALG.AP V-222 ALG.AP V-223	V _H	GAGGTGCAGCTGTTGGAG AGCGGGGGAGGCTTGGT ACAGCCTGGGGGGTCCCT GCGCCTCTCCTGTGCAGC CAGCGGATTCACCTTTTC TCACGGTTCTATGTACTG GGTCCGCCAGGCTCCAGG GAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGG TTCTGGTTCTACATACTA TGCAGACTCCGTGAAGGG CCGGTTCACCATCTCCCA TGACAATTCCAAGAACAC GCTGTATCTGCAAATGAA CAGCCTGCGTGCCGAGGA CACGGCTGTATATTATTG TGCGCGCTCTTCTTACTA CGGTTCTTACTACTCTATT GACTATTGGGGGCCAGGG AACCCTGGTCACCGTCTC CTCA	13	EVQLLESGGG LVQPGGSLRL SCAASGFTFSH GSMYWVRQA PGKGLEWVSS ISSGSGSTYYA DSVKGRFTISH DNSKNTLYLQ MNSLRAEDTA VYYCARSSYY GSYYSIDYWG QGTLVTVSS	14
	V _L	GACATCCAGATGACCCAG TCTCCATCCTCCCTGAGC GCATCTGTAGGAGACCGC GTCACCATCACTTGCCGG GCAAGTCAGAGCATTAGC AGCTATTTAAATTGGTAT CAGCAGAAACCAGGGAA AGCCCCTAAGCTCCTGAT CTATGCTGCATCCAGTTT GCAAAGTGGGGTCCCATC ACGTTTCAGTGGCAGTGG AAGCGGGACAGATTTAC TCTCACCATCAGCAGTCT ACAACCTGAAGATTTTGC AACTTATTACTGTCAACA GTACTACGACAACCTGCC CACTTTTGGCCAGGGGAC CAAGCTGGAGATCAAA	15	DIQMTQSPSSL SASVGDRVTIT CRASQSISSYL NWYQQKPGK APKLLIYAASS LQSGVPSRFSG SGSGTDFTLTI SSLQPEDFATY YCQQYYDNLP TFGQGTKLEIK	16
ALG.AP V-191	HCDR1	GGATTCACCTTTGATTAC GGTTCT	17	GFTFDYGS	18
	HCDR2	ATTTCTTCTGGTTCTGGTT CTACA	3	ISSGSGST	4

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
	HCDR3	GCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTG ACTAT	5	ARSSYYGSYY SIDY	6
	LCDR1	CAGAGCATTAGCAGCTAT	7	QSISSY	8
	LCDR2	GCTGCATCC	9	AAS	10
	LCDR3	CAACAGTACTACGACAAC CTGCCCACT	11	QQYYDNLPT	12
	V _H	GAGGTGCAGCTGTTGGAG AGCGGGGGAGGCTTGGT ACAGCCTGGGGGGTCCCT GCGCCTCTCCTGTGCAGC CAGCGGATTCACCTTTGA TTACGGTTCTATGTACTG GGTCCGCCAGGCTCCAGG GAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGG TTCTGGTTCTACATACTAT GCAGACTCCGTGAAGGGC CGGTTACCATCTCCAC GACAATTCCAAGAACACG CTGTATCTGCAAATGAAC AGCCTGCGTGCCGAGGAC ACGGCTGTATATTATTGT GCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTG ACTATTGGGGCCAGGGAA CCCTGGTCACCGTCTCCT CA	19	EVQLLESGGG LVQPGGSLRL SCAASGFTFD YGS MYWVRQ APGKGLEWVS SISSGSGSTYY ADSVKGRFTIS HDNSKNTLYL QMNSLRAEDT AVYYCARSSY YGSYYSIDYW GQGT LVT VSS	20
	V _L	GACATCCAGATGACCCAG TCTCCATCCTCCCTGAGC GCATCTGTAGGAGACCGC GTCACCATCACTTGCCGG GCAAGTCAGAGCATTAGC AGCTATTTAAATTGGTAT CAGCAGAAACCAGGGAA AGCCCCTAAGCTCCTGAT CTATGCTGCATCCAGTTT GCACAGTGGGGTCCCATC ACGTTTCAGTGGCAGTGG AAGCGGGACAGATTTTAC TCTCACCATCAGCAGTCT GCAACCTGAAGATTTTGC AACTTATTACTGTCAACA	21	DIQMTQSPSSL SASVGDRTIT CRASQSISSYL NWYQQKPGK APKLLIYAASS LHSGVPSRFSG SGSGTDFTLTI SSLQPEDFATY YCQQYYDNLPT TFGQGTKLEIK	22

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
		GTACTACGACAACCTGCC CACTTTTGGCCAGGGGAC CAAGCTGGAGATCAAA			
ALG.AP V-196	HCDR1	GGATTCACCTTTTCTTAC GGTTCT	23	GFTFSYGS	24
	HCDR2	ATTTCTTCTGGTTCTGGTT CTACA	3	ISSGSGST	4
	HCDR3	GCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTG ACTAT	5	ARSSYYGSYY SIDY	6
	LCDR1	CAGAGCATTAGCAGCTAT	7	QSISSY	8
	LCDR2	GCTGCATCC	9	AAS	10
	LCDR3	CAACAGTACTACGACAAC CTGCCCACT	11	QQYYDNLPT	12
	V _H	GAGGTGCAGCTGTTGGAG AGCGGGGGAGGCTTGGT ACAGCCTGGGGGGTCCCT GCGCCTCTCCTGTGCAGC CAGCGGATTCACCTTTTC TTACGGTTCTATGTACTG GGTCCGCCAGGCTCCAGG GAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGG TTCTGGTTCTACATACTAT GCAGACTCCGTGAAGGGC CGGTTACCATCTCCCAT GACAATTCCAAGAACACG CTGTATCTGCAAATGAAC AGCCTGCGTGCCGAGGAC ACGGCTGTATATTATTGT GCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTG ACTATTGGGGCCAGGGAA CCCTGGTCACCGTCTCCT CA	25	EVQLLESGGG LVQPGGSLRL SCAASGFTFSY GSMYWVRQA PGKGLEWVSS ISSGSGSTYYA DSVKGRFTISH DNSKNTLYLQ MNSLRAEDTA VYYCARSSYY GSYYSIDYWG QGTLVTVSS	26
	V _L	GACATCCAGATGACCCAG TCTCCATCCTCCCTGAGC GCATCTGTAGGAGACCGC GTCACCATCACTTGCCGG GCAAGTCAGAGCATTAGC AGCTATTAAATTGGTAT	15	DIQMTQSPSSL SASVGDRVTIT CRASQSISSYL NWYQQKPGK APKLLIYAASS LQSGVPSRFSG	16

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
		CAGCAGAAACCAGGGAA AGCCCCTAAGCTCCTGAT CTATGCTGCATCCAGTTT GCAAAGTGGGGTCCCATC ACGTTTCAGTGGCAGTGG AAGCGGGACAGATTTCAC TCTCACCATCAGCAGTCT ACAACCTGAAGATTTTGC AACTTATTACTGTCAACA GTACTACGACAACCTGCC CACTTTTGGCCAGGGGAC CAAGCTGGAGATCAAA		SGSGTDFTLTI SSLQPEDFATY YCQQYYDNLP TFGQGTKLEIK	
ALG.AP V-004 ALG.AP V-006 ALG.AP V-010 ALG.AP V-014 ALG.AP V-018	HCDR1	GGATTCACCTTTTCTTAC GGTTCT	23	GFTFSYGS	24
	HCDR2	ATTTCTTCTGGTTCTGGTT CTACA	3	ISSGSGST	4
	HCDR3	GCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTG ACTAT	5	ARSSYYGSYY SIDY	6
	LCDR1	CAGAGCATTAGCAGCTAT	7	QSISSY	8
	LCDR2	GCTGCATCC	9	AAS	10
	LCDR3	CAACAGTACTACGACAAC CTGCCCCACT	11	QQYYDNLP	12
	V _H	GAGGTGCAGCTGTTGGAG AGCGGGGGAGGCTTGGT ACAGCCTGGGGGGTCCCT GCGCCTCTCCTGTGCAGC CAGCGGATTCACCTTTTC TTACGGTTCTATGTACTG GGTCCGCCAGGCTCCAGG GAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGG TTCTGGTTCTACATACTAT GCAGACTCCGTGAAGGGC CGGTTACCATCTCCCGT GACAATTCCAAGAACACG CTGTATCTGCAAATGAAC AGCCTGCGTGCCGAGGAC ACGGCTGTATATTATTGT GCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTG ACTATTGGGGCCAGGGAA	27	EVQLLESGGG LVQPGGSLRL SCAASGFTFSY GSMYWVRQA PGKGLEWVSS ISSGSGSTYYA DSVKGRFTISR DNSKNTLYLQ MNSLRAEDTA VYYCARSSYY GSYYSIDYWG QGTLVTVSS	28

Construct	Component	DNA Sequence	DNA SEQ ID	AA sequence	AA SEQ ID
		CCCTGGTCACCGTCTCCTCA			
	V _L	GACATCCAGATGACCCAG TCTCCATCCTCCCTGAGC GCATCTGTAGGAGACCGC GTCACCATCACTTGCCGG GCAAGTCAGAGCATTAGC AGCTATTTAAATTGGTAT CAGCAGAAACCAGGGAA AGCCCCTAAGCTCCTGAT CTATGCTGCATCCAGTTT GCAAAGTGGGGTCCCATC ACGTTTCAGTGGCAGTGG AAGCGGGACAGATTTTAC TCTCACCATCAGCAGTCT GCAACCTGAAGATTTTGC AACTTATTACTGTCAACA GTACTACGACAACCTGCC CACTTTTGGCCAGGGGAC CAAGCTGGAGATCAAA	15	DIQMTQSPSSL SASVGDRVITIT CRASQSISSYL NWKYQQKPGK APKLLIYAASS LQSGVPSRFSG SGSGTDFTLTI SSLQPEDFATY YCQQYYDNLP TFGQGTKLEIK	16

[00209] In some embodiments, the present invention provides for multispecific polypeptides (*e.g.*, bispecific polypeptides) comprising a 4-1BB-binding domain. A multispecific 4-1BB-binding polypeptide or protein may comprise two binding domains (the domains can be designed to specifically bind the same or different targets), a hinge region, a linker (*e.g.*, a carboxyl-terminus or an amino-terminus linker), and an immunoglobulin constant region. A multispecific 4-1BB-binding protein may be a homodimeric protein comprising two identical, disulfide-bonded polypeptides. In some embodiments the 4-1BB binding domains may be derived from a monoclonal antibody that binds to 4-1BB (*e.g.*, Urelumab (BMS-66513) or PF-05082566 (Pfizer)), derived from a 4-1BB-binding domain described in PCT Application Publication No. WO 2016/185016, or derived from a 4-1BB-binding domain PCT Application No. PCT/EP2017/059656.

[00210] In some embodiments, a multispecific polypeptide comprising a 4-1BB binding domain may comprise, in order from amino-terminus to carboxyl-terminus: (i) a first binding domain; (ii) a hinge region; (iii) an immunoglobulin constant region; (iv) a binding domain linker; and (v) a 4-1BB-binding domain. In some embodiments, a multispecific polypeptide

comprising a 4-1BB binding domain may comprise, in order from amino-terminus to carboxyl-terminus: (i) a 4-1BB-binding domain; (ii) a binding domain linker; (iii) an immunoglobulin constant region; (iv) a hinge region; and (v) a first binding domain. Amino acid and nucleic acid sequences for exemplary anti-4-1BB binding domain sequences are provided in Table 7.

[00211] In certain embodiments, a 4-1BB-binding protein or polypeptide can comprise one or more additional binding domains (*e.g.*, second binding domain) that bind a target other than 4-1BB. These other binding domains can comprise, for example, a particular cytokine or a molecule that targets the binding domain polypeptide to, for example, a particular cell type, a toxin, an additional cell receptor, or an antibody.

[00212] In some embodiments of the disclosure, a 4-1BB-binding polypeptide is capable of forming a heterodimer with a second polypeptide chain and comprises a hinge region (a) immediately amino-terminal to an immunoglobulin constant region (*e.g.*, amino-terminal to a CH2 domain wherein the immunoglobulin constant region includes CH2 and CH3 domains, or amino-terminal to a CH3 domain wherein the immunoglobulin sub-regions includes CH3 and CH4 domains), (b) interposed between and connecting a binding domain (*e.g.*, scFv) and a immunoglobulin heterodimerization domain, (c) interposed between and connecting a immunoglobulin heterodimerization domain and an immunoglobulin constant region (*e.g.*, wherein the immunoglobulin constant region includes CH2 and CH3 domains or CH3 and CH4 domains), (d) interposed between and connecting an immunoglobulin constant region and a binding domain, (e) at the amino-terminus of a polypeptide chain, or (f) at the carboxyl-terminus of a polypeptide chain. A polypeptide chain comprising a hinge region as described herein will be capable of associating with a different polypeptide chain to form a heterodimeric protein provided herein, and the heterodimer formed will contain a binding domain that retains its target specificity or its specific target binding affinity.

[00213] In certain embodiments, a 4-1BB-binding polypeptide can comprise a target cell binding domain for recruitment of target cells to effector cells expressing 4-1BB. In certain embodiments, the target cell binding domain specifically binds to 5T4. In certain embodiments, a 4-1BB-binding protein as described herein can comprise (i) a binding domain that specifically binds 4-1BB and (ii) another binding domain that specifically binds to 5T4. Non-limiting examples of anti-5T4 antibodies from which the 5T4 binding domain can be derived include those described

in PCT Application Publication No. WO 2016/185016 and PCT Application No. PCT/EP2017/059656.

[00214] In some embodiments, the 4-1BB-binding polypeptide provided herein is a polypeptide comprising two scFvs. In some embodiments, the two scFvs comprise 4-1BB-binding domains. In certain embodiments, the two scFvs comprise identical 4-1BB-binding domains. In other embodiments, the two scFvs comprise different 4-1BB-binding domains. In other embodiments, the polypeptide comprises a 4-1BB-binding domain as a first scFv and an target cell binding domain as a second scFv. For example, the target cell binding domain may be an scFv specific for 5T4.

[00215] In some embodiments, the bispecific 4-1BB-binding polypeptides and proteins provided herein comprise an anti-4-1BB scFv that is at least about 82%, at least about 85%, at least about 87%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 110, 112, 114, and 116, wherein the polypeptide is a multispecific polypeptide in the format scFv-Fc-scFv and comprises a Y to H substitution in the HCDR1 at position 150 of the anti-4-1BB V_H and/or an R to H substitution in the FR3 at position 127 of the anti-4-1BB V_H. In certain embodiments, the bispecific 4-1BB-binding polypeptides and proteins provided herein comprise an anti-4-1BB scFv that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 110, 112, 114, and 116. Amino acid and nucleic acid sequences for exemplary anti-4-1BB scFvs are provided in Table 8 below.

Table 8. DNA and AA Sequences of Exemplary anti-4-1BB scFvs

Construct	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
ALG.AP V-178-4- 1BB	GAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCT GGGGGGTCCCTGCGCCTCTCCT GTGCAGCCAGCGGATTCACCT	109	EVQLLESGGGLVQPG GSLRLSCAASGFTFS HGSMYWVRQAPGK GLEWVSSISGSGSTY	110
ALG.AP V-179-4- 1BB	TTTCTCACGGTTCTATGTACTG GGTCCGCCAGGCTCCAGGGAA GGGGCTGGAGTGGGTCTCATC TATTTCTTCTGGTTCTGGTTCT ACATACTATGCAGACTCCGTG		YADSVKGRFTISHDN SKNTLYLQMNSLRAE DTAVYYCARSSYYG SYYSIDYWGQGTTLVT VSSGGGGSGGGGSG	

Construct	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
ALG.AP V-187-4-1BB	AAGGGCCGGTTCACCATCTCC CATGACAATTCCAAGAACACG CTGTATCTGCAAATGAACAGC CTGCGTGCCGAGGACACGGCT		GGGSGGGGSDIQMT QSPSSLSASVGDRVTI TCRASQSISSYLNWY QQKPGKAPKLLIYAA	
ALG.AP V-198-4-1BB	GTATATTATTGTGCGCGCTCTT CTTACTACGGTTCTTACTACTC TATTGACTATTGGGGCCAGGG AACCCTGGTCACCGTCTCCTCA		SSLQSGVPSRFSGSGS GTDFTLTISLQPEDF ATYYCQQYYDNLPT FGQGTKLEIK	
ALG.AP V-199-4-1BB	GGTGGAGGTGGCTCCGGGGGT GGAGGTTCCGGAGGAGGCGGA TCAGGTGGAGGCGGAAGCGAC ATCCAGATGACCCAGTCTCCA			
ALG.AP V-208-4-1BB	TCCTCCCTGAGCGCATCTGTAG GAGACCGCGTCACCATCACTT GCCGGGCAAGTCAGAGCATT GCAGCTATTTAAATTGGTATCA			
ALG.AP V-209-4-1BB	GCAGAAACCAGGGAAAGCCCC TAAGCTCCTGATCTATGCTGCA TCCAGTTTGCAAAGTGGGGTC CCATCACGTTTCAGTGGCAGT			
ALG.AP V-210-4-1BB	GGAAGCGGGACAGATTTCACT CTCACCATCAGCAGTCTACAA CCTGAAGATTTTGCAACTTATT ACTGTCAACAGTACTACGACA			
ALG.AP V-222-4-1BB	ACCTGCCCCACTTTTGGCCAGG GGACCAAGCTGGAGATCAAA			
ALG.AP V-223-4-1BB				
ALG.AP V-191-4-1BB	GAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCT GGGGGGTCCCTGCGCCTCTCCT GTGCAGCCAGCGGATTACCT TTGATTACGGTTCTATGTACTG GGTCCGCCAGGCTCCAGGGAA GGGGCTGGAGTGGGTCTCATC TATTTCTTCTGGTTCTGGTTCT ACATACTATGCAGACTCCGTG AAGGGCCGGTTCACCATCTCC CACGACAATTCCAAGAACACG CTGTATCTGCAAATGAACAGC	111	EVQLLES GGGLVQPG GSLRLSCAASGFTFD YGS MYWVRQAPGK GLEWVSSISSGSGSTY YADSVKGRFTISHDN SKNTLYLQMNSLRAE DTAVYYCARSSYYG SYYSIDYWGQGT LVT VSSGGGSGGGGSG GGGSGGGGSDIQMT QSPSSLSASVGDRV TI TCRASQSISSYLNWY	112

Construct	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
	CTGCGTGCCGAGGACACGGCT GTATATTATTGTGCGCGCTCTT CTTACTACGGTTCTTACTACTC TATTGACTATTGGGGCCAGGG AACCCTGGTCACCGTCTCCTCA GGTGGAGGTGGCTCCGGGGGT GGAGGTTCCGGAGGAGGCGGA TCAGGTGGAGGCGGAAGCGAC ATCCAGATGACCCAGTCTCCA TCCTCCCTGAGCGCATCTGTAG GAGACCGCGTCACCATCACTT GCCGGGCAAGTCAGAGCATT GCAGCTATTTAAATTGGTATCA GCAGAAACCAGGGAAAGCCCC TAAGCTCCTGATCTATGCTGCA TCCAGTTTGCACAGTGGGGTC CCATCACGTTTCAGTGGCAGT GGAAGCGGGACAGATTTCACT CTCACCATCAGCAGTCTGCAA CCTGAAGATTTTGCAACTTATT ACTGTCAACAGTACTACGACA ACCTGCCCCACTTTTGGCCAGG GGACCAAGCTGGAGATCAAA		QQKPGKAPKLLIYAA SSLHSGVPSRFSGSGS GTDFTLTISLQPEDF ATYYCQQYYDNLPT FGQGTKLEIK	
ALG.AP V-196-4- 1BB	GAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCT GGGGGGTCCCTGCGCCTCTCCT GTGCAGCCAGCGGATTCACCT TTTCTTACGGTTCTATGTACTG GGTCCGCCAGGCTCCAGGGAA GGGGCTGGAGTGGGTCTCATC TATTTCTTCTGGTTCTGGTTCT ACATACTATGCAGACTCCGTG AAGGGCCGGTTCACCATCTCC CATGACAATTCCAAGAACACG CTGTATCTGCAAATGAACAGC CTGCGTGCCGAGGACACGGCT GTATATTATTGTGCGCGCTCTT CTTACTACGGTTCTTACTACTC TATTGACTATTGGGGCCAGGG AACCCTGGTCACCGTCTCCTCA GGTGGAGGTGGCTCCGGGGGT GGAGGTTCCGGAGGAGGCGGA TCAGGTGGAGGCGGAAGCGAC	113	EVQLLES GGGLVQPG GSLRLSCAASGFTFS YGS MYWVRQAPGK GLEWVSSISSGSGSTY Y ADSVKGRFTISHDNS KNTLYLQMNSLRAE DTAVYYCARSSYYG SYYSIDYWGQGT LVT VS SGGGSGGGGSGGG GSGGGGSDIQMTQSP SSLSASVGD RVTITCR ASQSISSYLNWYQQK PGKAPKLLIYAASSL QSGVPSRFSGSGSGT DFTLTISLQPEDFAT YYCQQYYDNLPTFG QGTKLEIK	114

Construct	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
	ATCCAGATGACCCAGTCTCCA TCCTCCCTGAGCGCATCTGTAG GAGACCGCGTCACCATCACTT GCCGGGCAAGTCAGAGCATT GCAGCTATTTAAATTGGTATCA GCAGAAACCAGGGAAAGCCCC TAAGCTCCTGATCTATGCTGCA TCCAGTTTGCAAAGTGGGGTC CCATCACGTTTCAGTGGCAGT GGAAGCGGGACAGATTTCACT CTCACCATCAGCAGTCTACAA CCTGAAGATTTTGCAACTTATT ACTGTCAACAGTACTACGACA ACCTGCCCCACTTTTGGCCAGG GGACCAAGCTGGAGATCAAAA			
ALG.AP V-006-4- 1BB	GAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCT GGGGGGTCCCTGCGCCTCTCCT GTGCAGCCAGCGGATTCACCT	115	EVQLLESGGGLVQPG GSLRLSCAASGFTFS YGSMYWVRQAPGK GLEWVSSISSGSGSTY YADSVKGRFTISRDN SKNTLYLQMNSLRAE DTAVYYCARSSYYG SYYSIDYWGQGT LVTVSSGGGGSGGGGSG GGGSGGGGSDIQMT QSPSSLSASVGDRV TITCRASQSISSYLNWY QQKPGKAPKLLIYAA SSLQSGVPSRFSGS SGSGSTGDTFTLTIS SLQPEDFATYYCQQY YDNLPTFGQGTKLEIK	116
ALG.AP V-010-4- 1BB	TTTCTTACGGTTCTATGTACTG GGTCCGCCAGGCTCCAGGGAA GGGGCTGGAGTGGGTCTCATC TATTTCTTCTGGTTCTGGTTCT			
ALG.AP V-014-4- 1BB	ACATACTATGCAGACTCCGTG AAGGGCCGGTTCACCATCTCC CGTGACAATTCCAAGAACACG CTGTATCTGCAAATGAACAGC			
ALG.AP V-018-4- 1BB	CTGCGTGCCGAGGACACGGCT GTATATTATTGTGCGCGCTCTT CTTACTACGGTTCTTACTACTC TATTGACTATTGGGGCCAGGG AACCCTGGTCACCGTCTCCTCA GGCGGCGGCGGCAGCGGCGGC GGCGGCAGCGGCGGCGGAGGC TCCGGCGGCGGCGGCAGCGAC ATCCAGATGACCCAGTCTCCA TCCTCCCTGAGCGCATCTGTAG GAGACCGCGTCACCATCACTT GCCGGGCAAGTCAGAGCATT GCAGCTATTTAAATTGGTATCA GCAGAAACCAGGGAAAGCCCC TAAGCTCCTGATCTATGCTGCA TCCAGTTTGCAAAGTGGGGTC			

Construct	DNA Sequence	DNA SEQ ID	AA Sequence	AA SEQ ID
	CCATCACGTTTCAGTGGCAGT GGAAGCGGGACAGATTTCCT CTCACCATCAGCAGTCTGCAA CCTGAAGATTTTGCAACTTATT ACTGTCAACAGTACTACGACA ACCTGCCCCACTTTTGGCCAGG GGACCAAGCTGGAGATCAAA			

Bispecific molecules

[00216] In some embodiments, the present invention provides multispecific 5T4-binding polypeptides comprising two binding domains wherein one binding domain is specific for 5T4 and the second binding domain is specific for a different target antigen. In some embodiments, the present invention provides bispecific 4-1BB-binding polypeptides comprising two binding domains wherein binding domain is specific for 4-1BB and the second binding domain is specific for a different target antigen. In particular embodiments, the present invention provides bispecific polypeptides comprising two binding domains, wherein one binding domain is specific for 4-1BB and wherein the other binding domain is specific for 5T4. Such embodiments are referred to herein as anti-5T4 x anti-4-1BB molecules or anti-5T4 x anti-4-1BB polypeptides or proteins. In particular embodiments, the 5T4 and 4-1BB binding domains of an anti-5T4 x anti-4-1BB molecule are scFv domains that specifically bind to 5T4 and 4-1BB, respectively.

[00217] In some embodiments, the anti-5T4 x anti-4-1BB molecules described herein comprise in order from amino-terminus to carboxyl-terminus (i) a 5T4-binding domain; (ii) a binding domain linker; and (iii) a 4-1BB-binding domain. In some embodiments, the anti-5T4 x anti-4-1BB molecules described herein comprise in order from amino-terminus to carboxyl-terminus (i) a 4-1BB-binding domain; (ii) a binding domain linker; and (iii) a 5T4-binding domain. In some embodiments, the anti-5T4 x anti-4-1BB molecules described herein comprise in order from amino-terminus to carboxyl-terminus (or in order from carboxyl-terminus to amino-terminus), (i) a 5T4-binding domain, (ii) a hinge region, (iii) an immunoglobulin constant region, (iv) a carboxyl-terminus linker (or an amino-terminus linker), and (v) a second binding domain.

[00218] The anti-5T4 x anti-4-1BB molecules described herein may comprise any combination of the anti-5T4 and the anti-4-1BB binding domain sequences shown in Tables 4 – 7.

In particular embodiments, the anti-5T4 x anti-4-1BB molecules of the present invention comprise a 5T4-binding domain and a 4-1BB-binding domain that each comprise a HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3. In some embodiments, the anti-5T4 x anti-4-1BB molecules comprise (a) a first scFv domain comprising: (i) V_H comprising an HCDR1 amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 52, and 60, an HCDR2 amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 62, and an HCDR3 amino acid sequence of SEQ ID NO: 34; and (ii) a V_L comprising an LCDR1 amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 42, and 54, an LCDR2 amino acid sequence of SEQ ID NO: 10, and an LCDR3 amino acid sequence of SEQ ID NO: 36; and (b) a second scFv domain comprising (i) a V_H comprising an HCDR1 amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, and 24, an HCDR2 amino acid sequence of SEQ ID NO: 4, and an HCDR3 amino acid sequence of SEQ ID NO: 6; and (ii) a V_L comprising an LCDR1 amino acid sequence of SEQ ID NO: 8, an LCDR2 amino acid sequence of SEQ ID NO: 10, and an LCDR3 amino acid sequence of SEQ ID NO: 12.

[00219] In some embodiments, the anti-5T4 x anti-4-1BB molecules of the present invention comprise a 5T4-binding domain and a 4-1BB-binding domain that each comprise a V_H and a V_L domain. In some embodiments, the anti-5T4 x anti-4-1BB molecules comprise a first scFv domain comprising i) a V_H comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 38, 46, 56, and 64 and a V_L comprising an amino acid selected from the group consisting of SEQ ID NOs: 40, 44, 48, 50 58, 66, 68, and 70; and a second scFv domain comprising ii) a V_H comprising an amino acid selected from the group consisting of SEQ ID NO: 14, 20, 26, and 28 and a V_L comprising an amino acid selected from the group consisting of SEQ ID NO: 16 and 22.

[00220] In certain embodiments, the 5T4-binding domain of an anti-5T4 x anti-4-1BB molecule comprises or is an scFv that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 118, 120, 122, 124, 126, 128, 130, 132, 134, and 170, wherein the molecule is a multispecific polypeptide in the format scFv-Fc-scFv and comprises a Y to F substitution in the LCDR1 at position 99 of the anti-5T4 V_L and/or a F to S substitution in the FR3 at position 148 of the anti-5T4 V_L. In certain embodiments, the

5T4-binding domain of an anti-5T4 x anti-4-1BB molecule comprises an scFv that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 118, 120, 122, 124, 126, 128, 130, 132, 134, and 170.

[00221] In certain embodiments, the 4-1BB-binding domain of an anti-5T4 x anti-4-1BB molecule comprises or is an scFv that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 110, 112, 114, and 116, wherein the molecule is a multispecific polypeptide in the format scFv-Fc-scFv and comprises a Y to H substitution in the HCDR1 at position 150 of the anti-4-1BB V_H and/or an R to H substitution in the FR3 at position 127 of the anti-4-1BB V_H. In certain embodiments, the 4-1BB-binding domain of an anti-5T4 x anti-4-1BB molecule comprises scFv that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 110, 112, 114, and 116.

[00222] In particular embodiments, the anti-5T4 x anti-4-1BB molecule comprises a combination of CDR sequences, V_H/V_L sequences, and/or scFv sequences as shown in Table 9 below.

Table 9. Exemplary anti-5T4 and anti-4-1BB Binding Sequence Combinations

ALG.APV-178 (scFv-Fc-scFv Format)			
4-1BB Binding Domain		5T4 Binding Domain	
Component	SEQ ID NO:	Component	SEQ ID NO:
HCDR1	2	HCDR1	30
HCDR2	4	HCDR2	32
HCDR3	6	HCDR3	34
LCDR1	8	LCDR1	8
LCDR2	10	LCDR2	10
LCDR3	12	LCDR3	36
V_H	14	V_H	38
V_L	16	V_L	40
scFv	110	scFv	118
ALG.APV-179, ALG.APV-209, ALG.APV-222 (scFv-Fc-scFv Format)			
4-1BB Binding Domain		5T4 Binding Domain	
Component	SEQ ID NO:	Component	SEQ ID NO:
HCDR1	2	HCDR1	30
HCDR2	4	HCDR2	32
HCDR3	6	HCDR3	34

LCDR1	8	LCDR1	42
LCDR2	10	LCDR2	10
LCDR3	12	LCDR3	36
V_H	14	V_H	38
V_L	16	V_L	44
scFv	110	scFv	120
ALG.APV-187, ALG.APV-210, ALG.APV-223 (scFv-Fc-scFv Format)			
4-1BB Binding Domain		5T4 Binding Domain	
Component	SEQ ID NO:	Component	SEQ ID NO:
HCDR1	2	HCDR1	30
HCDR2	4	HCDR2	32
HCDR3	6	HCDR3	34
LCDR1	8	LCDR1	42
LCDR2	10	LCDR2	10
LCDR3	12	LCDR3	36
V_H	14	V_H	46
V_L	16	V_L	48
scFv	110	scFv	122
ALG.APV-191 (scFv-Fc-scFv Format)			
4-1BB Binding Domain		5T4 Binding Domain	
Component	SEQ ID NO:	Component	SEQ ID NO:
HCDR1	18	HCDR1	30
HCDR2	4	HCDR2	32
HCDR3	6	HCDR3	34
LCDR1	8	LCDR1	42
LCDR2	10	LCDR2	10
LCDR3	12	LCDR3	36
V_H	20	V_H	46
V_L	22	V_L	50
scFv	112	scFv	124
ALG.APV-196 (scFv-Fc-scFv Format)			
4-1BB Binding Domain		5T4 Binding Domain	
Component	SEQ ID NO:	Component	SEQ ID NO:
HCDR1	24	HCDR1	52
HCDR2	4	HCDR2	32
HCDR3	6	HCDR3	34
LCDR1	8	LCDR1	54
LCDR2	10	LCDR2	10
LCDR3	12	LCDR3	36
V_H	26	V_H	56
V_L	16	V_L	58
scFv	114	scFv	126
ALG.APV-198 (scFv-Fc-scFv Format)			
4-1BB Binding Domain		5T4 Binding Domain	

Component	SEQ ID NO:	Component	SEQ ID NO:
HCDR1	2	HCDR1	52
HCDR2	4	HCDR2	32
HCDR3	6	HCDR3	34
LCDR1	8	LCDR1	54
LCDR2	10	LCDR2	10
LCDR3	12	LCDR3	36
V _H	14	V _H	56
V _L	16	V _L	58
scFv	110	scFv	126
ALG.APV-199 (scFv-Fc-scFv Format)			
4-1BB Binding Domain		5T4 Binding Domain	
Component	SEQ ID NO:	Component	SEQ ID NO:
HCDR1	2	HCDR1	60
HCDR2	4	HCDR2	62
HCDR3	6	HCDR3	34
LCDR1	8	LCDR1	54
LCDR2	10	LCDR2	10
LCDR3	12	LCDR3	36
V _H	14	V _H	64
V _L	16	V _L	58
scFv	110	scFv	128
ALG.APV-006 (scFv-Fc-scFv Format)			
4-1BB Binding Domain		5T4 Binding Domain	
Component	SEQ ID NO:	Component	SEQ ID NO:
HCDR1	24	HCDR1	30
HCDR2	4	HCDR2	32
HCDR3	6	HCDR3	34
LCDR1	8	LCDR1	8
LCDR2	10	LCDR2	10
LCDR3	12	LCDR3	36
V _H	28	V _H	38
V _L	16	V _L	68
scFv	116	scFv	130
ALG.APV-010 (scFv-Fc-scFv Format)			
4-1BB Binding Domain		5T4 Binding Domain	
Component	SEQ ID NO:	Component	SEQ ID NO:
HCDR1	24	HCDR1	30
HCDR2	4	HCDR2	32
HCDR3	6	HCDR3	34
LCDR1	8	LCDR1	8
LCDR2	10	LCDR2	10
LCDR3	12	LCDR3	36
V _H	28	V _H	38
V _L	16	V _L	68

scFv	116	scFv	170
ALG.APV-014 (scFv-Fc-scFv Format)			
4-1BB Binding Domain		5T4 Binding Domain	
Component	SEQ ID NO:	Component	SEQ ID NO:
HCDR1	24	HCDR1	30
HCDR2	4	HCDR2	32
HCDR3	6	HCDR3	34
LCDR1	8	LCDR1	8
LCDR2	10	LCDR2	10
LCDR3	12	LCDR3	36
V_H	28	V_H	46
V_L	16	V_L	70
scFv	116	scFv	132
ALG.APV-018 (scFv-Fc-scFv Format)			
4-1BB Binding Domain		5T4 Binding Domain	
Component	SEQ ID NO:	Component	SEQ ID NO:
HCDR1	24	HCDR1	30
HCDR2	4	HCDR2	32
HCDR3	6	HCDR3	34
LCDR1	8	LCDR1	8
LCDR2	10	LCDR2	10
LCDR3	12	LCDR3	36
V_H	28	V_H	46
V_L	16	V_L	70
scFv	116	scFv	134

[00223] In some embodiments, the anti-5T4 x anti-4-1BB molecules described herein may comprise an amino acid sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 172, 174, and 176. In some embodiments, the anti-5T4 x anti-4-1BB molecules described herein may comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 172, 174, and 176. Exemplary amino acid and nucleic acid sequences for the anti-5T4 x anti-4-1BB molecules described herein are provided in Tables 10 and 11. In each of Tables 10 and 11, immunoglobulin Fc domains (hinge – CH1 – CH2) are indicated in underlined text, and binding domain linker sequences are indicated in bolded text.

Table 10. Exemplary anti-5T4 x anti-4-1BB Molecule DNA Sequences

Construct	DNA SEQ	DNA SEQ ID
ALG.APV-178	ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTACCTTTTCTCACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCATGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCT GGTCACCGTCTCCTCAGGTGGAGGTGGCTCCGGGGGTGGA GGTTCCGGAGGAGGCGGATCAGGTGGAGGCGGAAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTACAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAATCGAGT GAGCCCAAATCTTCTGACAAAACCTCACACATGCCCAACGT <u>GCCCAGCACCTGAAGCCGCGGGTGCACCGTCAGTCTTCCTC</u> <u>TTCCCCCCTAAACCAAGGACACCCTCATGATCTCCCGGAC</u> <u>CCCTGAGGTACATGCGTGGTGGTGACGTGAGCCACGAA</u> <u>GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG</u> <u>AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT</u> <u>ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG</u> <u>CACCAGGACTGGCTGAATGGCAAGGAATACAAGTGCGCGG</u> <u>TCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCAT</u> <u>CTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC</u> <u>ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGG</u> <u>TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGAC</u> <u>ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC</u> <u>AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT</u> <u>CCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG</u> <u>TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA</u> <u>GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT</u> <u>CTCCGGGTTCGGGAGGTGGCGGTTTCGGGAGGTGGCGGG</u> <u>TCAGGAGGTGGGGGATCCCCTTCAGAAGTGCAGCTGCTG</u> GAGTCCGGAGGAGGACTGGTGCAGCCTGGCGGAAGCCTGA GGCTGAGCTGCGCTGCCTCCGGCTTCACATTCAGCAGCTAT GCTATGAGCTGGGTGAGGCAAGCCCCTGGAAAGGGCCTGG	135

Construct	DNA SEQ	DNA SEQ ID
	AGTGGGTGTCCGCTATCTCCGGCAGCGGCGGAAGCACCTA CTACGCTGACTCCGTCAAGGGCAGGTTACCATCAGCCGG GACAACAGCAAGAACACCCTGTACCTGCAGATGAATAGCC TCAGGGCTGAAGACACCGCTGTGTACTACTGCGCCAGGTA CTATGGCGGCTACTACTCCGCCTGGATGGACTACTGGGGAC AGGGCACACTGGTGACCGTGTCCAGCGGCGGAGGCGGCTC CGGAGGCGGTGGCTCCGGAGGAGGCGGAAGCGGAGGAGG AGGCTCCGATATTCAGATGACACAGTCCCCTAGCTCCCTGT CCGCCAGCGTGCGGAGATCGGGTGACCATCACCTGCAGGGC CAGCCAGTCCATCTCCAGCTATTTAAACTGGTACCAGCAGA AGCCTGGAAAGGCTCCCAAGCTGCTGATCTACGCCGCTTCC AGCCTCCAGAGCGGCGTGCCTAGCAGGTTCTCCGGCTCCG GAAGCGGAACAGACTTCACCCTGACCATCAGCTCCCTGCA GCCCCAGGACTCCGCTACCTACTACTGCCAGCAGACCTATG GCTACCTGCACACCTTCGGCCAGGGCACAAAGCTGGAGAT CAAGCGC	
ALG.APV- 179	ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATAACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTCACCTTTTCTCACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCATGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCCT GGTCACCGTCTCCTCAGGTGGAGGTGGCTCCGGGGGTGGA GGTTCCGGAGGAGGCGGATCAGGTGGAGGCGGAAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTACAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAATCGAGT <u>GAGCCCAAATCTTCTGACAAAACCTCACACATGCCACCGT</u> <u>GCCCAGCACCTGAAGCCGCGGGTGACCGTCAGTCTTCCTC</u> <u>TTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGAC</u> <u>CCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAA</u> <u>GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG</u> <u>AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT</u> <u>ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG</u>	137

Construct	DNA SEQ	DNA SEQ ID
	<p> <u>CACCAGGACTGGCTGAATGGCAAGGAATACAAGTGC</u> <u>CGCGGTCTCCAACAAAGCCCCTCCCAGCCCCCATCGAGAAAACCAT</u> <u>CTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC</u> <u>ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGG</u> <u>TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGAC</u> <u>ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC</u> <u>AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT</u> <u>CCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG</u> <u>TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA</u> <u>GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT</u> <u>CTCCGGGT</u>TCCGGAGGTGGCGGTT<u>CGGGAGGTGGCGGG</u> TCAGGAGGTGGGGGATCCCCTTCAGAAGTGCAGCTGCTG GAGTCCGGAGGAGGACTGGTGCAGCCTGGCGGAAGCCTGA GGCTGAGCTGCGCTGCCTCCGGCTTCACATTCAGCAGCTAT GCTATGAGCTGGGTGAGGCAAGCCCCCTGGAAAGGGCCTGG AGTGGGTGTCCGCTATCTCCGGCAGCGGCGGAAGCACCTA CTACGCTGACTCCGTCAAGGGCAGGTTACCATCAGCCGG GACAACAGCAAGAACACCCTGTACCTGCAGATGAATAGCC TCAGGGCTGAAGACACCGCTGTGTACTACTGCGCCAGGTA CTATGGCGGCTACTACTCCGCCTGGATGGACTACTGGGGAC AGGGCACACTGGTGACCGTGTCCAGCGGCGGAGGCGGCTC CGGAGGCGGTGGCTCCGGAGGAGGCGGAAGCGGAGGAGG AGGCTCCGATATTCAGATGACACAGTCCCCTAGCTCCCTGT CCGCCAGCGTGGGAGATCGGGTGACCATCACCTGCAGGGC CAGCCAGTCCATCTCCAGCTTCTTAAACTGGTACCAGCAGA AGCCTGGAAAGGCTCCCAAGCTGCTGATCTACGCCGCTTCC AGCCTCCAGAGCGGCGTGCCTAGCAGGTTCTCCGGCTCCG GAAGCGGAACAGACTTCACCCTGACCATCAGCTCCCTGCA GCCCCGAGGACTCCGCTACCTACTACTGCCAGCAGACCTATG GCTACCTGCACACCTTCGGCCAGGGCACAAAGCTGGAGAT CAAGCGC </p>	
ALG.APV-187	<p> ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTCACCTTTTCTCACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCATGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCCT GGTCACCGTCTCCTCAGGTGGAGGTGGCTCCGGGGGTGGA GGTTCCGGAGGAGGCGGATCAGGTGGAGGCGGAAGCGAC </p>	139

Construct	DNA SEQ	DNA SEQ ID
	ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTACAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAATCGAGT GAGCCCAAATCTTCTGACAAAACCTCACACATGCCCACCGT <u>GCCCAGCACCTGAAGCCGCGGGTGCACCGTCAGTCTTCCTC</u> <u>TTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGAC</u> <u>CCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA</u> <u>GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG</u> <u>AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT</u> <u>ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG</u> <u>CACCAGGACTGGCTGAATGGCAAGGAATACAAGTGCGCGG</u> <u>TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCAT</u> <u>CTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC</u> <u>ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGG</u> <u>TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGAC</u> <u>ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC</u> <u>AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT</u> <u>CCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG</u> <u>TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA</u> <u>GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT</u> <u>CTCCGGGTTCGGGAGGTGGCGGTTTCGGGAGGTGGCGGG</u> <u>TCAGGAGGTGGGGGATCCCCTTCAGAAGTGACAGCTGCTG</u> GAGTCCGGAGGAGGACTGGTGCAGCCTGGCGGAAGCCTGA GGCTGAGCTGCGCTGCCTCCGGCTTCACATTCAGCAGCTAT GCTATGAGCTGGGTGAGGCAAGCCCCTGGAAAGTGCTGG AGTGGGTGTCCGCTATCTCCGGCAGCGGCGGAAGCACCTA CTACGCTGACTCCGTCAAGGGCAGGTTCACCATCAGCCGG GACAACAGCAAGAACACCCTGTACCTGCAGATGAATAGCC TCAGGGCTGAAGACACCGCTGTGTACTACTGCGCCAGGTA CTATGGCGGCTACTACTCCGCCTGGATGGACTACTGGGGAC AGGGCACACTGGTGACCGTGTCCAGCGGCGGAGGCGGCTC CGGAGGCGGTGGCTCCGGAGGAGGCGGAAGCGGAGGAGG AGGCTCCGATATTCAGATGACACAGTCCCCTAGCTCCCTGT CCGCCAGCGTGGGAGATCGGGTGACCATCACCTGCAGGGC CAGCCAGTCCATCTCCAGCTTCTTAAACTGGTACCAGCAGA AGCCTGGAAAGGCTCCCAAGCTGCTGATCTACGCCGCTTCC AGCCTCCAGAGCGGCGTGCCTAGCAGGTTCTCCGGCTCCG GAAGCGGAACAGACTTCACCCTGACCATCAGCTCCCTGCA	

Construct	DNA SEQ	DNA SEQ ID
	GCCCGAGGACTCCGCTACCTACTACTGCCAGCAGACCTATG GCTACCTGCACACCTTCGGCTGCGGCACAAAGCTGGAGAT CAAGCGC	
ALG.APV- 191	ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTACCTTTGATTACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCACGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCCT GGTCACCGTCTCCTCAGGTGGAGGTGGCTCCGGGGGTGGA GGTTCCGGAGGAGGCGGATCAGGTGGAGGCGGAAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAC AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAATCCTCG <u>GAGCCCAAATCTTCTGACAAAACCTCACACATGCCACCGT</u> <u>GCCCAGCACCTGAAGCCGCGGGTGCACCGTCAGTCTTCCTC</u> <u>TTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGAC</u> <u>CCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAA</u> <u>GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG</u> <u>AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT</u> <u>ACAACAGCACGTACCGTGTGGTCAGCGTCCTACCGTCCTG</u> <u>CACCAGGACTGGCTGAATGGCAAGGAATACAAGTGCGCGG</u> <u>TCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCAT</u> <u>CTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC</u> <u>ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGG</u> <u>TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGAC</u> <u>ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC</u> <u>AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT</u> <u>CCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG</u> <u>TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA</u> <u>GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT</u> <u>CTCCGGGTTCGGGAGGTGGCGGTTCCGGAGGTGGCGGG</u> <u>TCAGGAGGTGGGGGATCCCCTTCAGAAGTGCAGCTGCTG</u> <u>GAGTCCGGAGGAGGACTGGTGCAGCCTGGCGGAAGCCTGA</u>	141

Construct	DNA SEQ	DNA SEQ ID
	GGCTGAGCTGCGCTGCCTCCGGCTTCACATTCAGCAGCTAT GCTATGAGCTGGGTGAGGCAAGCCCCTGGAAAGTGCCTGG AGTGGGTGTCCGCTATCTCCGGCAGCGGCGGAAGCACCTA CTACGCTGACTCCGTCAAGGGCAGGTTACCATCAGCCGG GACAACAGCAAGAACACCCTGTACCTGCAGATGAATAGCC TCAGGGCTGAAGACACCGCTGTGTACTACTGCGCCAGGTA CTATGGCGGCTACTACTCCGCCTGGATGGACTACTGGGGAC AGGGCACACTGGTGACCGTGTCCAGCGGCGGAGGCGGCTC CGGAGGCGGTGGCTCCGGAGGAGGCGGAAGCGGAGGAGG AGGCTCCGATATTCAGATGACACAGTCCCCTAGCTCCCTGT CCGCCAGCGTGGGAGATCGGGTGACCATCACCTGCAGGGC CAGCCAGTCCATCTCCAGCTTCTTAAACTGGTACCAGCAGA AGCCTGGAAAGGCTCCCAAGCTGCTGATCTACGCCGCTTCC AGCCTCCAGAGCGGCGTGCCTAGCAGGTTCTCCGGCTCCG GAAGCGGAACAGACTTCACCCTGACCATCAGCTCCCTGCA GCCCCGAGGACTTCGCTACCTACTACTGCCAGCAGACCTATG GCTACCTGCACACCTTCGGCTGCGGCACAAAGCTGGAGAT CAAGAGC	
ALG.APV- 196	ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATAACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTACCTTTTCTTACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCATGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCT GGTCACCGTCTCCTCAGGTGGAGGTGGCTCCGGGGGTGGA GGTTCCGGAGGAGGCGGATCAGGTGGAGGCGGAAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTACAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAATCCTCG GAGCCCCAAATCTTCTGACAAAACCTCACACATGCCCAACGT <u>GCCCAGCACCTGAAGCCGCGGGTGCACCGTCAGTCTTCCTC</u> <u>TTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGAC</u> <u>CCCTGAGGTCAATGCGTGGTGGTGGACGTGAGCCACGAA</u> <u>GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG</u>	143

Construct	DNA SEQ	DNA SEQ ID
	<u>AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT</u> <u>ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG</u> <u>CACCAGGACTGGCTGAATGGCAAGGAATACAAGTGCGCGG</u> <u>TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCAT</u> <u>CTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC</u> <u>ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGG</u> <u>TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGAC</u> <u>ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC</u> <u>AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT</u> <u>CCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG</u> <u>TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA</u> <u>GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT</u> <u>CTCCGGGTTCGGGAGGTGGCGGTTCTGGGAGGTGGCGGG</u> <u>TCAGGAGGTGGGGGATCCCCTTCAGAAGTGACAGCTGCTG</u> GAGTCCGGAGGAGGACTGGTGCAGCCTGGCGGAAGCCTGA GGCTGAGCTGCGCTGCCTCCGGCTTCGACTTCGAGAGCTAT GCTATGAGCTGGGTGAGGCAAGCCCCTGGAAAGTGCTGG AGTGGGTGTCCGCTATCTCCGGCAGCGGCGGAAGCACCTA CTACGCTGACTCCGTCAAGGGCAGGTTACCATCAGCCGG GACAACAGCAAGAACACCCTGTACCTGCAGATGAATAGCC TCAGGGCTGAAGACACCGCTGTGTACTACTGCGCCAGGTA CTATGGCGGCTACTACTCCGCTGGATGGACTACTGGGGAC AGGGCACACTGGTGACCGTGTCCAGCGGCGGAGGCGGCTC CGGAGGCGGTGGCTCCGGAGGAGGCGGAAGCGGAGGAGG AGGCTCCGATATTCAGATGACACAGTCCCCTAGCTCCCTGT CCGCCAGCGTGAGGAGATCGGGTGACCATCACCTGCAGGGC CAGCCAGTCCATCAGGAGCGCCCTGAACTGGTACCAGCAG AAGCCTGGAAAGGCTCCCAAGCTGCTGATCTACGCCGCTTC CAGCCTCCAGAGCGGCGTGCCTAGCAGGTTCTCCGGCTCCG GAAGCGGAACAGACTTCACCCTGACCATCAGCTCCCTGCA GCCCCGAGGACTTCGCTACCTACTACTGCCAGCAGACCTATG GCTACCTGCACACCTTCGGCTGCGGCACAAAGCTGGAGAT CAAGCGC	
ALG.APV-198	ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTACCTTTTCTCACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCATGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCCT	145

Construct	DNA SEQ	DNA SEQ ID
	GGTCACCGTCTCCTCAGGTGGAGGTGGCTCCGGGGGTGGA GGTTCCGGAGGAGGCGGATCAGGTGGAGGCGGAAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTACAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAATCGAGT GAGCCCAAATCTTCTGACAAAACCTCACACATGCCACCGT <u>GCCCAGCACCTGAAGCCGCGGGTGCACCGTCAGTCTTCCTC</u> <u>TTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGAC</u> <u>CCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAA</u> <u>GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG</u> <u>AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT</u> <u>ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG</u> <u>CACCAGGACTGGCTGAATGGCAAGGAATACAAGTGCGCGG</u> <u>TCTCCAACAAGCCCTCCCAGCCCCATCGAGAAAACCAT</u> <u>CTCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC</u> <u>ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGG</u> <u>TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGAC</u> <u>ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC</u> <u>AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT</u> <u>CCTTCTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG</u> <u>TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA</u> <u>GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT</u> CTCCGGGT TCCGGAGGTGGCGGTTCCGGAGGTGGCGGG TCAGGAGGTGGGGGATCCCCTTC AGAAGTGCAGCTGCTG GAGTCCGGAGGAGGACTGGTGCAGCCTGGCGGAAGCCTGA GGCTGAGCTGCGCTGCCTCCGGCTTCGACTTCGAGAGCTAT GCTATGAGCTGGGTGAGGCAAGCCCCCTGGAAAGTGCCTGG AGTGGGTGTCCGCTATCTCCGGCAGCGGCGGAAGCACCTA CTACGCTGACTCCGTCAAGGGCAGGTTACCATCAGCCGG GACAACAGCAAGAACACCCTGTACCTGCAGATGAATAGCC TCAGGGCTGAAGACACCGCTGTGTACTACTGCGCCAGGTA CTATGGCGGCTACTACTCCGCCTGGATGGACTACTGGGGAC AGGGCACACTGGTGACCGTGTCCAGCGGCGGAGGCGGCTC CGGAGGCGGTGGCTCCGGAGGAGGCGGAAGCGGAGGAGG AGGCTCCGATATTCAGATGACACAGTCCCCTAGCTCCCTGT CCGCCAGCGTGGGAGATCGGGTGACCATCACCTGCAGGGC CAGCCAGTCCATCAGGAGCGCCCTGAACTGGTACCAGCAG AAGCCTGGAAAGGCTCCCAAGCTGCTGATCTACGCCGCTTC	

Construct	DNA SEQ	DNA SEQ ID
	CAGCCTCCAGAGCGGCGTGCCTAGCAGGTTCTCCGGCTCCG GAAGCGGAACAGACTTCACCCTGACCATCAGCTCCCTGCA GCCCCGAGGACTTCGCTACCTACTACTGCCAGCAGACCTATG GCTACCTGCACACCTTCGGCTGCGGCACAAAGCTGGAGAT CAAGCGC	
ALG.APV- 199	ATGGAAGCACCAAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTACCTTTTCTCACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCATGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCT GGTCACCGTCTCCTCAGGTGGAGGTGGCTCCGGGGGTGGA GGTTCCGGAGGAGGCGGATCAGGTGGAGGCGGAAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTACAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAATCGAGT <u>GAGCCCAAATCTTCTGACAAA</u> <u>ACTCACACATGCCACCGT</u> <u>GCCCAGCACCTGAAGCCGCGGGTGCACCGTCAGTCTTCCTC</u> <u>TTCCCCC</u> <u>AAAACCCAAGGACACCCTCATGATCTCCCGGAC</u> <u>CCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA</u> <u>GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG</u> <u>AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT</u> <u>ACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG</u> <u>CACCAGGACTGGCTGAATGGCAAGGAATACAAGTGCGCGG</u> <u>TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCAT</u> <u>CTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC</u> <u>ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGG</u> <u>TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGAC</u> <u>ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC</u> <u>AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT</u> <u>CCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG</u> <u>TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA</u> <u>GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT</u> <u>CTCCGGGT</u> <u>TCCGGAGGTGGCGGTTCCGGAGGTGGCGGG</u>	147

Construct	DNA SEQ	DNA SEQ ID
	TCAGGAGGTGGGGGATCCCCTTCAGAAGTGCAGCTGCTG GAGTCCGGAGGAGGACTGGTGCAGCCTGGCGGAAGCCTGA GGCTGAGCTGCGCTGCCTCCGGCTTCGACTTCGACAGCTAT GCTATGAGCTGGGTGAGGCAAGCCCCTGGAAAGTGCCTGG AGTGGGTGTCCGCTATCTCCGGCAGGGGCGGAAGCACCTA CTACGCTGACTCCGTCAAGGGCAGGTTACCATCAGCCGG GACAACAGCAAGAACACCCTGTACCTGCAGATGAATAGCC TCAGGGCTGAAGACACCGCTGTGTACTACTGCGCCAGGTA CTATGGCGGCTACTACTCCGCCTGGATGGACTACTGGGGAC AGGGCACACTGGTGACCGTGTCCAGCGGCGGAGGCGGCTC CGGAGGCGGTGGCTCCGGAGGAGGCGGAAGCGGAGGAGG AGGCTCCGATATTCAGATGACACAGTCCCCTAGCTCCCTGT CCGCCAGCGTGGGAGATCGGGTGACCATCACCTGCAGGGC CAGCCAGTCCATCAGGAGCGCCCTGAACTGGTACCAGCAG AAGCCTGGAAAGGCTCCCAAGCTGCTGATCTACGCCGCTTC CAGCCTCCAGAGCGGCGTGCCTAGCAGGTTCTCCGGCTCCG GAAGCGGAACAGACTTCACCCTGACCATCAGCTCCCTGCA GCCCCGAGGACTTCGCTACCTACTACTGCCAGCAGACCTATG GCTACCTGCACACCTTCGGCTGCGGCACAAAGCTGGAGAT CAAGCGC	
ALG.APV- 208	ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTCACCTTTTCTCACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCATGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCCT GGTCACCGTCTCCTCAGGTGGAGGTGGCTCCGGGGGTGGA GGTTCCGGAGGAGGCGGATCAGGTGGAGGCGGAAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTACAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAAGAGCCC <u>AAATCTTCTGACAAAACCTCACACATGCCACCGTGCCAGC</u> <u>ACCTGAAGCCGCGGGTGCACCGTCAGTCTTCCTCTTCCCC</u> <u>CAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA</u>	175

Construct	DNA SEQ	DNA SEQ ID
	GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCT GAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC ATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACA GCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAATGGCAAGGAATACAAGTGCGCGGTCTCCA ACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAA AGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG CCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCC TGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCC GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTAC AAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTT CCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCT GCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG GGTTCCGGAGGTGGCGGTTTCGGGAGGTGGCGGGTTCAG GAGGTGGGGGATCCCCTTC AGAAGTGCAGCTGCTGGAGT CCGGAGGAGGACTGGTGCAGCCTGGCGGAAGCCTGAGGCT GAGCTGCGCTGCCTCCGGCTTCACATTCAGCAGCTATGCTA TGAGCTGGGTGAGGCAAGCCCCTGGAAAGGGCCTGGAGTG GGTGTCCGCTATCTCCGGCAGCGGCGGAAGCACCTACTAC GCTGACTCCGTCAAGGGCAGGTTACCATCAGCCGGGACA ACAGCAAGAACACCCTGTACCTGCAGATGAATAGCCTCAG GGCTGAAGACACCGCTGTGTACTACTGCGCCAGGTACTAT GGCGGCTACTACTCCGCCTGGATGGACTACTGGGGACAGG GCACACTGGTGACCGTGTCCAGCGGCGGAGGCGGCTCCGG AGGCGGTGGCTCCGGAGGAGGCGGAAGCGGAGGAGGAGG CTCCGATATTCAGATGACACAGTCCCCTAGCTCCCTGTCCG CCAGCGTGGGAGATCGGGTGACCATCACCTGCAGGGCCAG CCAGTCCATCTCCAGCTATTTAAACTGGTACCAGCAGAAGC CTGGAAAGGCTCCCAAGCTGCTGATCTACGCCGCTTCCAGC CTCCAGAGCGGCGTGCCTAGCAGGTTCTCCGGCTCCGGAA GCGGAACAGACTTCACCCTGACCATCAGCTCCCTGCAGCCC GAGGACTCCGCTACCTACTACTGCCAGCAGACCTATGGCTA CCTGCACACCTTCGGCCAGGGCACAAAGCTGGAGATCAAG CGC	
ALG.APV-209 ALG.APV-222	ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATAACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTACCTTTTCTCACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCATGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG	171

Construct	DNA SEQ	DNA SEQ ID
	AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCCCT GGTCACCGTCTCCTCAGGTGGAGGTGGCTCCGGGGGTGGA GGTTCCGGAGGAGGCGGATCAGGTGGAGGCGGAAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTACAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAAGAGCCC AAATCTTCTGACAAAACCTCACACATGCCCACCGTGCCCAGC ACCTGAAGCCGCGGGTGCACCGTCAGTCTTCTCTTCCCCC CAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCT GAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC ATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACA GCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAATGGCAAGGAATACAAGTGCGCGGTCTCCA ACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAA AGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG CCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCC TGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCC GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTAC AAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTT CCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCT GCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG GGTTCCGGAGGTGGCGGTTTCGGGAGGTGGCGGGTTCAG GAGGTGGGGGATCCCCCTTCAGAAGTGCAGCTGCTGGAGT CCGGAGGAGGACTGGTGCAGCCTGGCGGAAGCCTGAGGCT GAGCTGCGCTGCCTCCGGCTTCACATTCAGCAGCTATGCTA TGAGCTGGGTGAGGCAAGCCCCTGGAAAGGGCCTGGAGTG GGTGTCCGCTATCTCCGGCAGCGGCGGAAGCACCTACTAC GCTGACTCCGTCAAGGGCAGGTTACCATCAGCCGGGACA ACAGCAAGAACACCCTGTACCTGCAGATGAATAGCCTCAG GGCTGAAGACACCGCTGTGTACTACTGCGCCAGGTACTAT GGCGGCTACTACTCCGCCTGGATGGACTACTGGGGACAGG GCACACTGGTGACCGTGTCCAGCGGCGGAGGCGGCTCCGG AGGCGGTGGCTCCGGAGGAGGCGGAAGCGGAGGAGGAGG CTCCGATATTCAGATGACACAGTCCCCTAGCTCCCTGTCCG CCAGCGTGGGAGATCGGGTGACCATCACCTGCAGGGCCAG	

Construct	DNA SEQ	DNA SEQ ID
	CCAGTCCATCTCCAGCTTCTTAAACTGGTACCAGCAGAAGC CTGGAAAGGCTCCCAAGCTGCTGATCTACGCCGCTTCCAGC CTCCAGAGCGGCGTGCCTAGCAGGTTCTCCGGCTCCGGAA GCGGAACAGACTTCACCCTGACCATCAGCTCCCTGCAGCCC GAGGACTCCGCTACCTACTACTGCCAGCAGACCTATGGCTA CCTGCACACCTTCGGCCAGGGCACAAAGCTGGAGATCAAG CGC	
ALG.APV- 210 ALG.APV- 223	ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTACCTTTTCTCACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCATGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCCT GGTCACCGTCTCCTCAGGTGGAGGTGGCTCCGGGGGTGGA GGTTCCGGAGGAGGCGGATCAGGTGGAGGCGGAAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTACAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAAGAGCCC <u>AAATCTTCTGACAAAACCTCACACATGCCACCGTGCCCAGC</u> <u>ACCTGAAGCCGCGGGTGCACCGTCAGTCTTCCTCTTCCCC</u> <u>CAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA</u> <u>GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCT</u> <u>GAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC</u> <u>ATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACA</u> <u>GCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG</u> <u>GA CTGGCTGAATGGCAAGGAATACAAGTGCGCGGTCTCCA</u> <u>ACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAA</u> <u>AGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG</u> <u>CCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCC</u> <u>TGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCC</u> <u>GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTAC</u> <u>AAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTT</u> <u>CCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG</u> <u>CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCT</u>	173

Construct	DNA SEQ	DNA SEQ ID
	<u>GCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG</u> <u>GGTTCCGGAGGTGGCGGTTTCGGGAGGTGGCGGGTCAG</u> GAGGTGGGGGATCCCCCTTCAGAAGTGCAGCTGCTGGAGT CCGGAGGAGGACTGGTGCAGCCTGGCGGAAGCCTGAGGCT GAGCTGCGCTGCCTCCGGCTTCACATTCAGCAGCTATGCTA TGAGCTGGGTGAGGCAAGCCCCTGGAAAGTGCCTGGAGTG GGTGTCCGCTATCTCCGGCAGCGGCGGAAGCACCTACTAC GCTGACTCCGTCAAGGGCAGGTTACCATCAGCCGGGACA ACAGCAAGAACACCCTGTACCTGCAGATGAATAGCCTCAG GGCTGAAGACACCGCTGTGTACTACTGCGCCAGGTACTAT GGCGGCTACTACTCCGCCTGGATGGACTACTGGGGACAGG GCACACTGGTGACCGTGTCCAGCGGCGGAGGCGGCTCCGG AGGCGGTGGCTCCGGAGGAGGCGGAAGCGGAGGAGGAGG CTCCGATATTCAGATGACACAGTCCCCTAGCTCCCTGTCCG CCAGCGTGGGAGATCGGGTGACCATCACCTGCAGGGCCAG CCAGTCCATCTCCAGCTTCTTAAACTGGTACCAGCAGAAGC CTGGAAAGGCTCCCAAGCTGCTGATCTACGCCGCTTCCAGC CTCCAGAGCGGCGTGCCTAGCAGGTTCTCCGGCTCCGGAA GCGGAACAGACTTCACCCTGACCATCAGCTCCCTGCAGCCC GAGGACTCCGCTACCTACTACTGCCAGCAGACCTATGGCTA CCTGCACACCTTCGGCTGCGGCACAAAGCTGGAGATCAAG CGC	
ALG.APV-006	ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATAACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTCACCTTTTCTTACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCGTGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCCT GGTCACCGTCTCCTCAGGCGGCGGCGGCAGCGGCGGCGGC GGCAGCGGCGGCGGAGGCTCCGGCGGCGGCGGCAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAATCCTCG AGTGAGCCCAAATCTTCTGACAAAACCTCACACATGCCAC	149

Construct	DNA SEQ	DNA SEQ ID
	<u>CGTGCCCAGCACCTGAAGCCGCGGGTGCACCGTCAGTCTTC</u> <u>CTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCG</u> <u>GACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC</u> <u>GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG</u> <u>TGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC</u> <u>AGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC</u> <u>CTGCACCAGGACTGGCTGAATGGCAAGGAATACAAGTGCG</u> <u>CGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAAC</u> <u>CATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG</u> <u>TACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACC</u> <u>AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGC</u> <u>GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG</u> <u>AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACG</u> <u>GCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGC</u> <u>AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC</u> <u>ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTC</u> <u>CCTGTCTCCGGGTTCGGGAGGTGGCGGTTTCGGGAGGTG</u> <u>GCGGGTCAGGAGGTGGGGGATCCCCTTCAGAGGTGCAG</u> CTGTTGGAGAGCGGGGGAGGCTTGGTACAGCCTGGGGGGT CCCTGCGCCTCTCCTGTGCAGCCAGCGGATTCACCTTTAGC AGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGG GGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAG CACATACTATGCAGACTCCGTGAAGGGCCGGTTCACCATCT CCCGTGACAATTCCAAGAACACGCTGTATCTGCAAATGAA CAGCCTGCGTGCCGAGGACACGGCTGTATATTATTGTGCGC GCTACTACGGTGGTTACTACTCTGCTTGGATGGACTATTGG GGCCAGGGAACCCTGGTCACCGTCTCCTCAGGCGGTGGAG GCAGCGGTGGGGGTGGGTCTGGAGGCGGTGGCAGTGGCGG CGGAGGCTCTGACATCCAGATGACCCAGTCTCCATCCTCCC TGAGCGCATCTGTAGGAGACCGCGTCACCATCACTTGCCG GGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAG CAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTG CATCCAGTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGC AGTGGAAGCGGGACAGATTTCACTCTCACCATCAGCAGTC TGCAACCTGAAGATTTTGCAACTTATTACTGTCAACAGACT TACGGTTACCTGCACACTTTTGGCCAGGGGACCAAGCTGG AGATCAAATCA	
ALG.APV-010	ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTCACCTTTTCTTACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG	151

Construct	DNA SEQ	DNA SEQ ID
	ACTCCGTGAAGGGCCGGTTCACCATCTCCCGTGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCCT GGTCACCGTCTCCTCAGGCGGCGGCGGCAGCGGCGGCGGC GGCAGCGGCGGCGGAGGCTCCGGCGGCGGCGGCAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAATCCTCG AGTGAGCCCAAATCTTCTGACAAAACTCACACATGCCCCAC <u>CGTGCCCAGCACCTGAAGCCGCGGGTGCACCGTCAGTCTTC</u> <u>CTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCG</u> <u>GACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC</u> <u>GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG</u> <u>TGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC</u> <u>AGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC</u> <u>CTGCACCAGGACTGGCTGAATGGCAAGGAATACAAGTGCG</u> <u>CGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAAC</u> <u>CATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG</u> <u>TACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACC</u> <u>AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGC</u> <u>GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG</u> <u>AACA ACTACAAGACCACGCTCCCGTGCTGGACTCCGACG</u> <u>GCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGC</u> <u>AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC</u> <u>ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTC</u> <u>CCTGTCTCCGGGTTC CGGAGGTGGCGGTTCGGGAGGTG</u> GCGGGTCAGGAGGTGGGGGATCCCCTTCAGACATCCAG ATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGTAGGAGA CCGCGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGC AGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCC CTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGG GTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGACAGATT TCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCA ACTTATTACTGTCAACAGACTTACGGTTACCTGCACACTTT TGGCCAGGGGACCAAGCTGGAGATCAAAGGCGGTGGAGG CAGCGGTGGGGGTGGGTCTGGAGGCGGTGGCAGTGGCGGC GGAGGCTCTGAGGTGCAGCTGTTGGAGAGCGGGGGAGGCT	

Construct	DNA SEQ	DNA SEQ ID
	TGGTACAGCCTGGGGGGTCCCTGCGCCTCTCCTGTGCAGCC AGCGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCG CCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATT AGTGGTAGTGGTGGTAGCACATACTATGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCCGTGACAATTCCAAGAACAC GCTGTATCTGCAAATGAACAGCCTGCGTGCCGAGGACACG GCTGTATATTATTGTGCGCGCTACTACGGTGGTTACTACTC TGCTTGGATGGACTATTGGGGCCAGGGAACCCTGGTCACC GTCTCCTCA	
ALG.APV- 014	ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATAACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTCACCTTTTCTTACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCGTGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCCT GGTCACCGTCTCCTCAGGCGGCGGCGGCAGCGGCGGCGGC GGCAGCGGCGGCGGAGGCTCCGGCGGCGGCGGCAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAATCCTCG AGTGAGCCCAAATCTTCTGACAAAACCTCACACATGCCAC <u>CGTGCCCAGCACCTGAAGCCGCGGGTGACCGTCAGTCTTC</u> <u>CTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCG</u> <u>GACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC</u> <u>GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG</u> <u>TGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC</u> <u>AGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC</u> <u>CTGCACCAGGACTGGCTGAATGGCAAGGAATACAAGTGCG</u> <u>CGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAAC</u> <u>CATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG</u> <u>TACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACC</u> <u>AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGC</u> <u>GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG</u> <u>AACA ACTACAAGACCACGCCTCCCGTGCTGGACTCCGACG</u>	153

Construct	DNA SEQ	DNA SEQ ID
	<p> <u>GCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGC</u> <u>AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC</u> <u>ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTC</u> <u>CCTGTCTCCGGGTTCGGGAGGTGGCGGTTCGGGAGGTG</u> <u>GCGGGTCAGGAGGTGGGGGATCCCCTTCAGAGGTGCAG</u> CTGTTGGAGAGCGGGGGAGGCTTGGTACAGCCTGGGGGGT CCCTGCGCCTCTCCTGTGCAGCCAGCGGATTACCTTTAGC AGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGT GCCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGC ACATACTATGCAGACTCCGTGAAGGGCCGGTTCACCATCTC CCGTGACAATTCCAAGAACACGCTGTATCTGCAAATGAAC AGCCTGCGTGCCGAGGACACGGCTGTATATTATTGTGCGCG CTACTACGGTGGTTACTACTCTGCTTGGATGGACTATTGGG GCCAGGGAACCCTGGTCACCGTCTCCTCAGGCGGTGGAGG CAGCGGTGGGGGTGGGTCTGGAGGCGGTGGCAGTGGCGGC GGAGGCTCTGACATCCAGATGACCCAGTCTCCATCCTCCCT GAGCGCATCTGTAGGAGACCGCGTCACCATCACTTGCCGG GCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGC AGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGC ATCCAGTTTGCAAAGTGGGGTCCCATCACGTTTCAGTGGCA GTGGAAGCGGGACAGATTTCACTCTCACCATCAGCAGTCT GCAACCTGAAGATTTTGCAACTTATTACTGTCAACAGACTT ACGGTTACCTGCACACTTTTGGCTGCGGGACCAAGCTGGA GATCAAATCA </p>	
ALG.APV-018	<p> ATGGAAGCACCAGCGCAGCTTCTCTTCCTCCTGCTACTCTG GCTCCCAGATAACCACCGGTGAGGTGCAGCTGTTGGAGAGC GGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTCT CCTGTGCAGCCAGCGGATTACCTTTTCTTACGGTTCTATG TACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG TCTCATCTATTTCTTCTGGTTCTGGTTCTACATACTATGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCCGTGACAATTCC AAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCG AGGACACGGCTGTATATTATTGTGCGCGCTCTTCTTACTAC GGTTCTTACTACTCTATTGACTATTGGGGCCAGGGAACCCT GGTCACCGTCTCCTCAGGCGGCGGCGGCAGCGGCGGCGGC GGCAGCGGCGGCGGAGGCTCCGGCGGCGGCGGCAGCGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGT AGGAGACCGCGTCACCATCACTTGCCGGGCAAGTCAGAGC ATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA AGTGGGGTCCCATCACGTTTCAGTGGCAGTGAAGCGGGA CAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGAT TTTGCAACTTATTACTGTCAACAGTACTACGACAACCTGCC </p>	155

Construct	DNA SEQ	DNA SEQ ID
	<p> CACTTTTGGCCAGGGGACCAAGCTGGAGATCAAATCCTCG AGTGAGCCCAAATCTTCTGACAAAACCTCACACATGCCCAC CGTGCCCAGCACCTGAAGCCGCGGGTGCACCGTCAGTCTTC CTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCG GACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCAC GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG TGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC AGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC CTGCACCAGGACTGGCTGAATGGCAAGGAATACAAGTGCG CGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAAC CATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG TACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACC AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGC GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACG GCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGC AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTC CCTGTCTCCGGGTTCCGGAGGTGGCGGTTCGGGAGGTG GCGGGTCAGGAGGTGGGGGATCCCCTTCAGACATCCAG ATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGTAGGAGA CCGCGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGC AGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCC CTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGG GTCCCATCACGTTTCAGTGGCAGTGGAAGCGGGACAGATT TCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCA ACTTATTACTGTCAACAGACTTACGGTTACCTGCACACTTT TGGCTGCGGGACCAAGCTGGAGATCAAAGGCGGTGGAGGC AGCGGTGGGGGTGGGTCTGGAGGCGGTGGCAGTGGCGGCG GAGGCTCTGAGGTGCAGCTGTTGGAGAGCGGGGGAGGCTT GGTACAGCCTGGGGGGTCCCTGCGCCTCTCCTGTGCAGCCA GCGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGC CAGGCTCCAGGGAAGTGCCTGGAGTGGGTCTCAGCTATTA GTGGTAGTGGTGGTAGCACATACTATGCAGACTCCGTGAA GGGCCGGTTCACCATCTCCCGTGACAATTCCAAGAACACG CTGTATCTGCAAATGAACAGCCTGCGTGCCGAGGACACGG CTGTATATTATTGTGCGCGCTACTACGGTGGTTACTACTCT GCTTGATGGACTATTGGGGCCAGGGAACCCTGGTCACCG TCTCCTCA </p>	

Table 11. Exemplary anti-5T4 x anti-4-1BB Molecule Amino Acid Sequences

Construct	AA Sequence	AA SEQ ID
ALG.APV-178	MEAPAQLLFLLLLWLPDTTGEVQLLESGGGLVQPGGSLRLSC AASGFTFSHGSMYWVRQAPGKGLEWVSSISSGSGSTYYADSV KGRFTISHDNSKNTLYLQMNSLRAEDTAVYYCARSSYYGSYY SIDYWGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQ SPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYY DNLPTFGQGTKLEIKSSEPKSSDKTHTCPPCPAPEAAGAPSVFL <u>FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV</u> <u>HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVS</u> <u>NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC</u> <u>LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL</u> <u>TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGG</u> SGGGGSGGGGSPSEVQLLESGGGLVQPGGSLRLSCAASGFTF SSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDY WGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSS LSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISLQPEDSATYYCQQTYGYLH TFGQGTKLEIKR	136
ALG.APV-179	MEAPAQLLFLLLLWLPDTTGEVQLLESGGGLVQPGGSLRLSC AASGFTFSHGSMYWVRQAPGKGLEWVSSISSGSGSTYYADSV KGRFTISHDNSKNTLYLQMNSLRAEDTAVYYCARSSYYGSYY SIDYWGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQ SPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYY DNLPTFGQGTKLEIKSSEPKSSDKTHTCPPCPAPEAAGAPSVFL <u>FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV</u> <u>HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVS</u> <u>NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC</u> <u>LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL</u> <u>TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGG</u> SGGGGSGGGGSPSEVQLLESGGGLVQPGGSLRLSCAASGFTF SSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDY WGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSS LSASVGDRVTITCRASQSISSFLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISLQPEDSATYYCQQTYGYLH TFGQGTKLEIKR	138
ALG.APV-187	MEAPAQLLFLLLLWLPDTTGEVQLLESGGGLVQPGGSLRLSC AASGFTFSHGSMYWVRQAPGKGLEWVSSISSGSGSTYYADSV KGRFTISHDNSKNTLYLQMNSLRAEDTAVYYCARSSYYGSYY SIDYWGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQ	140

Construct	AA Sequence	AA SEQ ID
	SPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYY DNLPTFGQGTKLEIKS <u>SEPKSSDKTHTCPPCPAPEAAGAPSVFL</u> <u>FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV</u> <u>HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVS</u> <u>NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC</u> <u>LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL</u> <u>TVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGSGGGG</u> <u>SGGGGSGGGGSPSEVQLLES</u> GGGLVQPGGSLRLSCAASGFTF SSYAMSWVRQAPGKCLEWVSAISGSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDY WGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSS LSASVGDRVTITCRASQSISSFLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDSATYYCQQTYGYLH TFGCGTKLEIKR	
ALG.APV-191	MEAPAQLLFLLLLWLPD TTGEVQLLES GGGLVQPGGSLRLSC AASGFTFDYGS MYWVRQAPGKGLEWVSSISSGSGSTYYADS VKGRFTISHDNSKNTLYLQMNSLRAEDTAVYYCARSSYYGSY YSIDYWGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSDIQMT QSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI YAASSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQY YDNLPTFGQGTKLEIKS <u>SEPKSSDKTHTCPPCPAPEAAGAPSVF</u> <u>LFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVE</u> <u>VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAV</u> <u>SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC</u> <u>LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL</u> <u>TVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGSGGGG</u> <u>SGGGGSGGGGSPSEVQLLES</u> GGGLVQPGGSLRLSCAASGFTF SSYAMSWVRQAPGKCLEWVSAISGSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDY WGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSS LSASVGDRVTITCRASQSISSFLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQTYGYLH TFGCGTKLEIKS	142
ALG.APV-196	MEAPAQLLFLLLLWLPD TTGEVQLLES GGGLVQPGGSLRLSC AASGFTFSYGS MYWVRQAPGKGLEWVSSISSGSGSTYYADSV KGRFTISHDNSKNTLYLQMNSLRAEDTAVYYCARSSYYGSYY SIDYWGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQ SPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYY DNLPTFGQGTKLEIKS <u>SEPKSSDKTHTCPPCPAPEAAGAPSVFL</u> <u>FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV</u> <u>HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVS</u>	144

Construct	AA Sequence	AA SEQ ID
	<u>NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGSGGGGSGGGGSPSEVQLLES</u> GGGLVQPGGSLRLSCAASGFD ESYAMSWVRQAPGKCLEWVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSIR SALN WYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQTYGYLHTFGCGTKLEIKR	
ALG.APV-198	MEAPAQLLFLLLLWLPD TTGEVQLLESGGGLVQPGGSLRLSCAASGFTFSHGSMYWVRQAPGKGLEWVSSISSGSGSTYYADSVKGRFTISHDN SKNTLYLQMNSLRAEDTAVYYCARSSYYGSYYSIDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQYYDNLPTFGQGTKLEIKSSEPKSSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGSGGGGSGGGGSPSEVQLLESGGGLVQPGGSLRLSCAASGFD ESYAMSWVRQAPGKCLEWVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSIR SALN WYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQTYGYLHTFGCGTKLEIKR	146
ALG.APV-199	MEAPAQLLFLLLLWLPD TTGEVQLLESGGGLVQPGGSLRLSCAASGFTFSHGSMYWVRQAPGKGLEWVSSISSGSGSTYYADSVKGRFTISHDN SKNTLYLQMNSLRAEDTAVYYCARSSYYGSYYSIDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQYYDNLPTFGQGTKLEIKSSEPKSSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGSGGGGSGGGGSPSEVQLLESGGGLVQPGGSLRLSCAASGFD DSYAMSWVRQAPGKCLEWVSAISGRGGSTYYADSVKGRFTI	148

Construct	AA Sequence	AA SEQ ID
	SRDNSKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDYWGQGTTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSIRSNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTYGYLHTFGCGTKLEIKR	
ALG.APV-208	MEAPAQLLFLLLLWLPDPTTGEVQLLES GGGLVQPGGSLRLSCAASGFTFSHGSMYWVRQAPGKGLEWVSSISSGSGSTYYADSVKGRFTISHDNSKNTLYLQMNSLRAEDTAVYYCARSSYYGSYYSIDYWGQGTTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYYDNLPTFGQGTKLEIKEPKSSDKTHTCPPCPAPEAAGAPSVFLFPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGSGGGGSGGGGSGGGGSPSEVQLLES GGGLVQPGGSLRLSCAASGFTFSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDYWGQGTTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDSATYYCQQTYGYLHTFGQGTKLEIKR	176
ALG.APV-209 ALG.APV-222	MEAPAQLLFLLLLWLPDPTTGEVQLLES GGGLVQPGGSLRLSCAASGFTFSHGSMYWVRQAPGKGLEWVSSISSGSGSTYYADSVKGRFTISHDNSKNTLYLQMNSLRAEDTAVYYCARSSYYGSYYSIDYWGQGTTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYYDNLPTFGQGTKLEIKEPKSSDKTHTCPPCPAPEAAGAPSVFLFPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGSGGGGSGGGGSGGGGSPSEVQLLES GGGLVQPGGSLRLSCAASGFTFSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDYWGQGTTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSFLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDSATYYCQQTYGYLHTFGQGTKLEIKR	172

Construct	AA Sequence	AA SEQ ID
ALG.APV-210 ALG.APV-223	MEAPAQLLFLLLLWLPDTTGEVQLLES GGGLVQPGGSLRLSC AASGFTFSHGSMYWVRQAPGKGLEWVSSISSGSGSTYYADSV KGRFTISHDNSKNTLYLQMNSLRAEDTAVYYCARSSYYGSYY SIDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQ SPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYY DNLPTFGQGTKLEI <u>KEPKSSDKTHTCPPCPAPEAAGAPSVFLFP</u> <u>PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH</u> <u>NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSN</u> <u>KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL</u> <u>VKGFYPSDIAVEWESNGOPENNYKTTTPVLDSDGSFFLYSKLT</u> <u>VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGS</u> GGGGSGGGGSP EVQLLES GGGLVQPGGSLRLSCAASGFTFS SYAMSWVRQAPGKCLEWVSAISGSGGSTYYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDYW GQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLS ASVGDRVTITCRASQSISSFLNWYQQKPGKAPKLLIYAASSLQ SGVPSRFSGSGSGTDFTLTISSLQPEDSATYYCQQTYGYLHTF GCGTKLEIKR	174
ALG.APV-006	MEAPAQLLFLLLLWLPDTTGEVQLLES GGGLVQPGGSLRLSC AASGFTFSYGS MYWVRQAPGKGLEWVSSISSGSGSTYYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARSSYYGSYY SIDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQ SPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYY DNLPTFGQGTKLEIKSSSEP <u>KSSDKTHTCPPCPAPEAAGAPSVF</u> <u>LFPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE</u> <u>VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAV</u> <u>SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC</u> <u>L VKGFYPSDIAVEWESNGOPENNYKTTTPVLDSDGSFFLYSKL</u> <u>TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGG</u> SGGGSGGGGSP EVQLLES GGGLVQPGGSLRLSCAASGFTF SSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTIS RDN SKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDY WGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSS LSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQTYGYLH TFGQGTKLEIKS	150
ALG.APV-010	MEAPAQLLFLLLLWLPDTTGEVQLLES GGGLVQPGGSLRLSC AASGFTFSYGS MYWVRQAPGKGLEWVSSISSGSGSTYYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARSSYYGSYY SIDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQ SPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY	152

Construct	AA Sequence	AA SEQ ID
	AASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYY DNLPTFGQGTKLEIKSSSEPKSSDKTHTCPPCPAPEAAGAPSVF <u>LFPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE</u> <u>VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAV</u> <u>SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC</u> <u>LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL</u> <u>TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGG</u> <u>SGGGGSGGGGSPSDIQMTQSPSSLSASVGDRVTITCRASQSI</u> SYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTL TISLQPEDFATYYCQQTYGYLHTFGQGTKLEIKGGGGSGGG GSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFS SYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCARYYGGYSAWMDYW GQGTLVTVSS	
ALG.APV-014	MEAPAQLLFLLLLWLPD TTGEVQLLES GGGLVQPGGSLRLSC AASGFTFSYGSMYWVRQAPGKGLEWVSSISSGSGSTYYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARSSYYGSYY SIDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQ SPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYY DNLPTFGQGTKLEIKSSSEPKSSDKTHTCPPCPAPEAAGAPSVF <u>LFPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE</u> <u>VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAV</u> <u>SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC</u> <u>LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL</u> <u>TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGG</u> <u>SGGGGSGGGGSPSEVQLLES GGGLVQPGGSLRLSCAASGFTF</u> SSYAMSWVRQAPGKCLEWVSAISGSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARYYGGYSAWMDY WGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSS LSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTYGYLH TFGCGTKLEIKS	154
ALG.APV-018	MEAPAQLLFLLLLWLPD TTGEVQLLES GGGLVQPGGSLRLSC AASGFTFSYGSMYWVRQAPGKGLEWVSSISSGSGSTYYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARSSYYGSYY SIDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQ SPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYY DNLPTFGQGTKLEIKSSSEPKSSDKTHTCPPCPAPEAAGAPSVF <u>LFPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE</u> <u>VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAV</u> <u>SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC</u>	156

Construct	AA Sequence	AA SEQ ID
	LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGG SGGGGSGGGGSPSDIQMTQSPSSLSASVGDRVTITCRASQIS SYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQTYGYLHTFGCGTKLEIKGGGGSGGG GSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFS SYAMSWVRQAPGKCLEWVSAISGSGGSTYYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCARYYGGYSAWMDYW GQGTLVTVSS	

Polynucleotides and Methods of Protein Expression

[00224] The disclosure also includes nucleic acids (*e.g.*, DNA or RNA) encoding the polypeptides of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) or one or more polypeptide chains of a polypeptide as described herein. Nucleic acids of the disclosure include nucleic acids having a region that is substantially identical to a polynucleotide as listed in Tables 1 – 10, *infra*. In certain embodiments, a nucleic acid in accordance with the present disclosure has at least 80%, typically at least about 90%, and more typically at least about 95% or at least about 98% identity to a polypeptide-encoding polynucleotide as listed in Tables 1 – 10, wherein the nucleic acid encodes a polypeptide that is a multispecific polypeptide in the format scFv-Fc-scFv and wherein the encoded polypeptide comprises one or more of (1) a Y to F substitution in the LCDR1 at position 99 of the anti-5T4 V_L; (2) a F to S substitution in the FR3 at position 148 of the anti-5T4 V_L; (3) a Y to H substitution in the HCDR1 at position 150 of the anti-4-1BB V_H; and (4) an R to H substitution in the FR3 at position 127 of the anti-4-1BB V_H. Nucleic acids of the disclosure also include complementary nucleic acids. In some instances, the sequences will be fully complementary (no mismatches) when aligned. In other instances, there can be up to about a 20% mismatch in the sequences. In some embodiments of the disclosure are provided nucleic acids encoding both first and second polypeptide chains of a bispecific protein of the disclosure. The nucleic acid sequences provided herein can be exploited using codon optimization, degenerate sequence, silent mutations, and other DNA techniques to optimize expression in a particular host, and the present disclosure encompasses such sequence modifications.

[00225] The disclosure relates to an isolated nucleic acid molecule encoding polypeptides of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof), wherein said nucleic acid molecule comprises a nucleotide sequence selected from SEQ ID NOs: 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 171, 173, and 175.

[00226] Polynucleotide molecules comprising a desired polynucleotide sequence are propagated by placing the molecule in a vector. Viral and non-viral vectors are used, including plasmids. The choice of plasmid will depend on the type of cell in which propagation is desired and the purpose of propagation. Certain vectors are useful for amplifying and making large amounts of the desired DNA sequence. Other vectors are suitable for expression in cells in culture. Still other vectors are suitable for transfer and expression in cells in a whole animal or person. The choice of appropriate vector is well within the skill of the art. Many such vectors are available commercially. The partial or full-length polynucleotide is inserted into a vector typically by means of DNA ligase attachment to a cleaved restriction enzyme site in the vector. Alternatively, the desired nucleotide sequence can be inserted by homologous recombination *in vivo*. Typically this is accomplished by attaching regions of homology to the vector on the flanks of the desired nucleotide sequence. Regions of homology are added by ligation of oligonucleotides, or by polymerase chain reaction using primers comprising both the region of homology and a portion of the desired nucleotide sequence, for example.

[00227] For expression, an expression cassette or system may be employed. To express a nucleic acid encoding a polypeptide disclosed herein, a nucleic acid molecule encoding the polypeptide, operably linked to regulatory sequences that control transcriptional expression in an expression vector, is introduced into a host cell. In addition to transcriptional regulatory sequences, such as promoters and enhancers, expression vectors can include translational regulatory sequences and a marker gene which is suitable for selection of cells that carry the expression vector. The gene product encoded by a polynucleotide of the disclosure is expressed in any convenient expression system, including, for example, bacterial, yeast, insect, amphibian and mammalian systems. In the expression vector, the polypeptide-encoding polynucleotide is linked to a regulatory sequence as appropriate to obtain the desired expression properties. These can include promoters, enhancers, terminators, operators, repressors, and inducers. The promoters can be regulated (*e.g.*, the promoter

from the steroid inducible pIND vector (Invitrogen)) or constitutive (*e.g.*, promoters from CMV, SV40, Elongation Factor, or LTR sequences). These are linked to the desired nucleotide sequence using the techniques described above for linkage to vectors. Any techniques known in the art can be used. Accordingly, the expression vector will generally provide a transcriptional and translational initiation region, which can be inducible or constitutive, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region.

[00228] An expression cassette (“expression unit”) can be introduced into a variety of vectors, *e.g.*, plasmid, BAC, YAC, bacteriophage such as lambda, P1, M13, *etc.*, plant or animal viral vectors (*e.g.*, retroviral-based vectors, adenovirus vectors), and the like, where the vectors are normally characterized by the ability to provide selection of cells comprising the expression vectors. The vectors can provide for extrachromosomal maintenance, particularly as plasmids or viruses, or for integration into the host chromosome. Where extrachromosomal maintenance is desired, an origin sequence is provided for the replication of the plasmid, which can be low- or high copy-number. A wide variety of markers are available for selection, particularly those which protect against toxins, more particularly against antibiotics. The particular marker that is chosen is selected in accordance with the nature of the host, where, in some cases, complementation can be employed with auxotrophic hosts. Introduction of the DNA construct can use any convenient method, including, *e.g.*, conjugation, bacterial transformation, calcium-precipitated DNA, electroporation, fusion, transfection, infection with viral vectors, biolistics, and the like. The disclosure relates to an expression vector comprising a nucleic acid segment, wherein said nucleic acid segment may comprise a nucleotide sequence selected from SEQ ID NOs: 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 171, 173, and 175.

[00229] Accordingly, proteins for use within the present disclosure can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells (including cultured cells of multicellular organisms), particularly cultured mammalian cells. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (3rd ed., Cold Spring Harbor

Laboratory Press, Cold Spring Harbor, NY, 2001), and Ausubel *et al.*, *Short Protocols in Molecular Biology* (4th ed., John Wiley & Sons, 1999).

[00230] For example, for recombinant expression of a homodimeric binding protein comprising two identical binding polypeptides as described herein, an expression vector will generally include a nucleic acid segment encoding the binding polypeptide, operably linked to a promoter. For recombinant expression of a heterodimeric binding protein comprising different first and second polypeptide chains, the first and second polypeptide chains can be co-expressed from separate vectors in the host cell for expression of the entire heterodimeric protein. Alternatively, for the expression of heterodimeric binding proteins the first and second polypeptide chains are co-expressed from separate expression units in the same vector in the host cell for expression of the entire heterodimeric protein. The expression vector(s) are transferred to a host cell by conventional techniques, and the transfected cells are then cultured by conventional techniques to produce the encoded polypeptide(s) to produce the corresponding binding proteins (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof).

[00231] To direct a recombinant protein into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence) is provided in the expression vector. The secretory signal sequence can be that of the native form of the recombinant protein, or can be derived from another secreted protein or synthesized *de novo*. The secretory signal sequence is operably linked to the polypeptide-encoding DNA sequence, *i.e.*, the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences can be positioned elsewhere in the DNA sequence of interest (*see, e.g.*, U.S. Patent Nos. 5,037,743 and 5,143,830).

[00232] Cultured mammalian cells are suitable hosts for production of recombinant polypeptides and proteins of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) for use within the present disclosure. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler *et al.*, Cell 14:725, 1978; Corsaro and Pearson, Somatic Cell Genetics 7:603, 1981; Graham and Van der Eb, Virology 52:456, 1973), electroporation (Neumann *et al.*, EMBO J. 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel *et*

al., *supra*), and liposome-mediated transfection (Hawley-Nelson *et al.*, Focus 15:73, 1993; Ciccarone *et al.*, Focus 15:80, 1993). The production of recombinant polypeptides in cultured mammalian cells is disclosed by, for example, U.S. Patent Nos. 4,713,339; 4,784,950; 4,579,821; and 4,656,134. Examples of suitable mammalian host cells include African green monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61; CHO DG44; CHO DXB11 (Hyclone, Logan, UT); *see also, e.g.*, Chasin *et al.*, *Som. Cell. Molec. Genet.* 12:555, 1986)), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658). Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Manassas, Virginia. Strong transcription promoters can be used, such as promoters from SV-40 or cytomegalovirus. *See, e.g.*, U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patents Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

[00233] Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as “transfectants.” Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as “stable transfectants.” Exemplary selectable markers include a gene encoding resistance to the antibiotic neomycin, which allows selection to be carried out in the presence of a neomycin-type drug, such as G-418 or the like; the gpt gene for xanthine-guanine phosphoribosyl transferase, which permits host cell growth in the presence of mycophenolic acid/xanthine; and markers that provide resistance to zeocin, bleomycin, blastocidin, and hygromycin (*see, e.g.*, Gatignol *et al.*, *Mol. Gen. Genet.* 207:342, 1987; Drocourt *et al.*, *Nucl. Acids Res.* 18:4009, 1990). Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as “amplification.” Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. An exemplary amplifiable selectable marker is dihydrofolate reductase,

which confers resistance to methotrexate. Other drug resistance genes (*e.g.*, hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used.

[00234] Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar *et al.*, *J. Biosci. (Bangalore)* 11:47-58, 1987. Transformation of insect cells and production of foreign polypeptides therein is disclosed in U.S. Patent No. 5,162,222 and PCT Publication No. WO 94/06463.

[00235] Insect cells can be infected with recombinant baculovirus, commonly derived from *Autographa californica* nuclear polyhedrosis virus (AcNPV). *See* King and Possee, *The Baculovirus Expression System: A Laboratory Guide* (Chapman & Hall, London); O'Reilly *et al.*, *Baculovirus Expression Vectors: A Laboratory Manual* (Oxford University Press., New York 1994); and *Baculovirus Expression Protocols. Methods in Molecular Biology* (Richardson ed., Humana Press, Totowa, NJ, 1995). Recombinant baculovirus can also be produced through the use of a transposon-based system described by Luckow *et al.* (*J. Virol.* 67:4566-4579, 1993). This system, which utilizes transfer vectors, is commercially available in kit form (BAC-TO-BAC kit; Life Technologies, Gaithersburg, MD). The transfer vector (*e.g.*, PFASTBAC1; Life Technologies) contains a Tn7 transposon to move the DNA encoding the protein of interest into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." *See* Hill-Perkins and Possee, *J. Gen. Virol.* 71:971-976, 1990; Bonning *et al.*, *J. Gen. Virol.* 75:1551-1556, 1994; and Chazenbalk and Rapoport, *J. Biol. Chem.* 270:1543-1549, 1995. In addition, transfer vectors can include an in-frame fusion with DNA encoding a polypeptide extension or affinity tag as disclosed above. Using techniques known in the art, a transfer vector containing a protein-encoding DNA sequence is transformed into *E. coli* host cells, and the cells are screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, such as Sf9 cells. Recombinant virus that expresses the protein or interest is subsequently produced. Recombinant viral stocks are made by methods commonly used in the art.

[00236] For protein production, the recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *Spodoptera frugiperda* (*e.g.*, Sf9 or Sf21 cells) or *Trichoplusia ni* (*e.g.*, HIGH FIVE™ cells; Invitrogen, Carlsbad, CA). *See generally* Glick

and Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA* (ASM Press, Washington, D.C., 1994). *See also* U.S. Patent No. 5,300,435. Serum-free media are used to grow and maintain the cells. Suitable media formulations are known in the art and can be obtained from commercial suppliers. The cells are grown up from an inoculation density of approximately $2\text{--}5 \times 10^5$ cells to a density of $1\text{--}2 \times 10^6$ cells, at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. Procedures used are generally described in available laboratory manuals (*see, e.g.,* King and Possee, *supra*; O'Reilly *et al., supra*; Richardson, *supra*).

[00237] Fungal cells, including yeast cells, can also be used within the present disclosure to produce the polypeptides of the present disclosure (*e.g.,* 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof). Yeast species of in this regard include, *e.g., Saccharomyces cerevisiae, Pichia pastoris, and Pichia methanolica*. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, U.S. Patent Nos. 4,599,311; 4,931,373; 4,870,008; 5,037,743; and 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (*e.g.,* leucine). An exemplary vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (*see, e.g.,* U.S. Patent Nos. 4,599,311; 4,615,974; and 4,977,092) and alcohol dehydrogenase genes. *See also* U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936; and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guilliermondii, and Candida maltosa* are known in the art. *See, e.g.,* Gleeson *et al., J. Gen. Microbiol.* 132:3459-3465, 1986; U.S. Patent No. 4,882,279; and Raymond *et al., Yeast* 14:11-23, 1998. *Aspergillus* cells can be utilized according to the methods of McKnight *et al.,* U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.,* U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533. Production of recombinant proteins in *Pichia methanolica* is disclosed in U.S. Patents Nos. 5,716,808; 5,736,383; 5,854,039; and 5,888,768.

[00238] Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus*, and other genera are also useful host cells within the present disclosure to produce, for example, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof including anti-5T4 x anti-4-1BB molecules. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well-known in the art (*see, e.g.,* Sambrook and Russell, *supra*). When expressing a recombinant protein in bacteria such as *E. coli*, the protein can be retained in the cytoplasm, typically as insoluble granules, or can be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured protein can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the alternative, the protein can be recovered from the cytoplasm in soluble form and isolated without the use of denaturants. The protein is recovered from the cell as an aqueous extract in, for example, phosphate buffered saline. To capture the protein of interest, the extract is applied directly to a chromatographic medium, such as an immobilized antibody or heparin-Sepharose column. Secreted proteins can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding. Antibodies, including single-chain antibodies, can be produced in bacterial host cells according to known methods. *See, e.g.,* Bird *et al.*, *Science* 242:423-426, 1988; Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883, 1988; and Pantoliano *et al.*, *Biochem.* 30:10117-10125, 1991.

[00239] Transformed or transfected host cells to produce the polypeptides and proteins of the present disclosure (*e.g.,* 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media can also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added

DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

[00240] The proteins and polypeptides of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) may be purified by conventional protein purification methods, typically by a combination of chromatographic techniques. *See generally Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988); Scopes, *Protein Purification: Principles and Practice* (Springer-Verlag, New York 1994). Proteins comprising an immunoglobulin Fc region can be purified by affinity chromatography on immobilized protein A or protein G. Additional purification steps, such as gel filtration, can be used to obtain the desired level of purity or to provide for desalting, buffer exchange, and the like.

Compositions and Methods of Use

[00241] The present disclosure provides methods for treating a subject with a disorder characterized by expression of 5T4. Generally, such methods include administering to a subject in need of such treatment a protein the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof). In some embodiments, a 5T4-binding polypeptide, 4-1BB-binding polypeptide, and/or multispecific binding proteins thereof (*e.g.*, an anti-5T4 x anti-4-1BB molecule) does not induce or induces minimal antibody-dependent cell-mediated cytotoxicity (ADCC) activity and/or complement-dependent cytotoxicity (CDC) activity.

[00242] In other embodiments, where the binding proteins (*e.g.*, multispecific binding proteins) comprise a second binding domain that specifically binds an effector cell (*e.g.*, to 4-1BB), the binding proteins and/or multispecific binding proteins result in enhanced effector cell activation against 5T4-expressing cells in the subject. For example, in some embodiments, the binding proteins and/or multispecific binding proteins result in increased effector cell proliferation, increased effector cell production of one or more cytokines (*e.g.*, IFN γ , TNF α , IL-6, IL-8, IL-12, IL-1 α , etc.), or increased expression of one or more cell-surface activation markers (*e.g.*, CD137, MHC-II, or CD69). Activation of effector cells can be measured by a variety of means known in the art including flow cytometry, immunofluorescence, and immunohistochemistry to assess

changes in cell-surface marker expression, ELISA to assess production of cytokines and other factors, cell counts to assess proliferation, qPCR to assess changes in gene expression, and the like.

[00243] In some embodiments, the multi-specific binding proteins described herein exhibit enhanced effector cell activation compared to a second multispecific polypeptide of a different structure. For example, in some embodiments, the first multispecific polypeptide comprises a first scFv domain that specifically binds to 5T4 and a second scFv that specifically binds to 4-1BB, together by a binding domain linker and an immunoglobulin Fc domain in the following configuration, from amino-terminus to carboxyl-terminus: (i) the first scFv domain, (ii) the hinge region, (iii) the immunoglobulin constant region, (iv) the binding domain linker, and (v) the second scFv domain. In this example, a second multispecific binding protein comprises an IgG-scFv structure comprising an anti-4-1BB antibody and an anti-5T4 scFv. In some embodiments, the first multispecific binding protein exhibits a statistically significant enhancement of effector cell activation compared to the second multispecific polypeptide. For example, in some embodiments, the first multispecific binding protein exhibits a lower EC₅₀ value in a Jurkat/NF-κB reporter cell assay than observed for the second multispecific binding protein (*See e.g.*, Example 10). In some embodiments, the EC₅₀ of the first multispecific binding protein is decreased by about 2 fold, about 3 fold, about 4 fold, about 5 fold, about 6 fold, about 7 fold, about 8 fold, about 9 fold, about 10 fold, or more compared to the EC₅₀ of the second multispecific binding protein. EC₅₀ values can be determined by non-linear regression analysis and other statistical methods known in the art.

[00244] In some embodiments, the first multispecific binding protein induces a statistically significant increase in effector cell proliferation compared to the second multispecific polypeptide. For example, in some embodiments, the first multispecific binding protein induces a statistically significant increase in proliferation of primed, human CD8⁺ T cells compared to the proliferation induced by the second multispecific binding protein (*See e.g.*, Example 22). In some embodiments, the first multispecific binding protein induces an increase in effector cell proliferation of about 2 fold, about 3 fold, about 4 fold, about 5 fold, about 6 fold, about 7 fold, about 8 fold, about 9 fold, about 10 fold, or more compared to the effector cell proliferation induced by the the second multispecific binding protein.

[00245] In certain variations of the method of treating a subject with a protein the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or

multispecific binding proteins thereof), the disorder is a cancer. Exemplary cancers amenable to treatment in accordance with a protein the present disclosure include, for example, breast cancer (*e.g.*, triple negative breast cancer (TNBC)), pancreatic cancer, ovarian cancer, lung cancer (*e.g.*, non-small cell lung cancer), hematologic malignancies (*e.g.*, chronic lymphocytic leukemia (CLL), mantle cell leukemia (MCL) or acute lymphoblastic leukemia (ALL)), skin cancer (*e.g.*, squamous cell carcinoma or melanoma), adrenal cancer, bladder cancer, cervical cancer, renal cancer, gastric cancer, prostate cancer, thyroid cancer, liver cancer, uterine cancer, a tumor formed on a nerve cell or nerve cell sheath (*e.g.*, neurofibroma), sarcoma, carcinoma or head and neck cancer. TNBC is defined as breast cancer with the absence of staining for estrogen receptor, progesterone receptor, and *HER2/neu*. In some embodiments, a protein of the present disclosure can be administered to a subject to treat mesothelioma in the subject. In one embodiment, a protein of the present disclosure can be administered to a subject to treat a clear cell carcinoma in the subject. In one embodiment, a protein of the present disclosure can be administered to a subject to treat a striated muscle tumor in the subject.

[00246] In a further embodiment, the disclosure encompasses a method for enhancing effector cell activation against a cell expressing 5T4, the method comprising contacting said 5T4-expressing cell with a protein of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof), wherein said contacting is under conditions whereby enhanced effector cell activation against the 5T4-expressing cell is induced. In some embodiments, the disclosure relates to a method for enhancing effector cell activation against a cell expressing 5T4, the method comprising: contacting said 5T4-expressing cell with a protein of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) comprising a first binding domain that specifically binds an epitope of human 5T4 and a second binding domain that specifically binds 4-1BB (*e.g.*, an anti-5T4 x anti-4-1BB molecule); wherein said contacting is under conditions whereby enhanced effector cell activation against the 5T4-expressing cell is induced. The disclosure encompasses a method for inducing effector cell dependent lysis of a cell expressing 5T4, the method comprising: contacting said 5T4-expressing cell with a protein of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof), wherein the second binding domain specifically binds 4-1BB (*e.g.*, an anti-5T4 x anti-4-1BB molecule); and wherein said contacting is under conditions

whereby enhanced effector cell activation of the 5T4-expressing cell is induced, thereby resulting in the lysis of the 5T4-expressing cell. In some embodiments, the disclosure relates to a method for inducing effector cell dependent lysis of a cell expressing 5T4, the method comprising: contacting said 5T4-expressing cell with a protein of the present disclosure comprising a first binding domain that specifically binds an epitope of human 5T4 and a second binding domain that specifically binds 4-1BB (*e.g.*, an anti-5T4 x anti-4-1BB molecule); wherein said contacting is under conditions whereby enhanced effector cell activation of the 5T4-expressing cell is induced thereby resulting the lysis of the 5T4-expressing cell.

[00247] The disclosure also encompasses proteins and polypeptides (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) for the manufacture of a medicament for treatment of a disorder (*e.g.*, cancer) characterized by expression of 5T4. In one embodiment, the protein or polypeptide comprises an anti-5T4 and anti-4-1BB binding domain (*e.g.*, an anti-5T4 x anti-4-1BB molecule) and has enhanced effector cell activation activity. In one embodiment, the disclosure provides proteins and polypeptides (*e.g.*, an anti-5T4 x anti-4-1BB molecule) for use in treating a disorder (*e.g.*, cancer) characterized by expression of 5T4. In certain embodiments, the disclosure relates to a method for treating a disorder in a subject, wherein said disorder is characterized by expression of 5T4, the method comprising administering to the subject a therapeutically effective amount of a protein or polypeptide of the present disclosure comprising a 5T4 binding domain that specifically binds an epitope of human 5T4 (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof).

[00248] In some embodiments, the disclosure provides a method of treating a patient with a cancer, comprising administering to the patient a polypeptide comprising amino acid sequence set forth in herein (*e.g.*, an amino acid sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 172, 174, and 176). In further embodiments, the polypeptide comprises an Fc. For example, in some embodiments, the disclosure provides a method of treating a patient with a cancer, comprising administering to the patient a polypeptide comprising a 5T4-binding domain and an Fc, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 172, 174, and 176. In still further embodiments, the disclosure provides a method of treating a patient with a cancer comprising administering to the patient a

polypeptide comprising a 5T4-binding domain and a 4-1BB-binding domain, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 172, 174, and 176. In one embodiment, the disclosure provides a method of treating a patient with a cancer comprising administering to the patient a polypeptide comprising a 5T4-binding domain and a 4-1BB-binding domain, wherein the polypeptide comprises SEQ ID NO: 172. In one embodiment, the disclosure provides a method of treating a patient with a cancer comprising administering to the patient a polypeptide comprising a 5T4-binding domain and a 4-1BB-binding domain, wherein the polypeptide comprises SEQ ID NO: 174.

[00249] In some embodiments, for treatment methods and uses described herein, a protein or polypeptide described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) is delivered in a manner consistent with conventional methodologies associated with management of the disease or disorder for which treatment is sought. In accordance with the disclosure herein, a therapeutically effective amount of the protein or polypeptide is administered to a subject in need of such treatment for a time and under conditions sufficient to prevent or treat the disease or disorder.

[00250] Subjects for administration of a protein of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) include patients at high risk for developing a particular disorder characterized by 5T4 expression as well as patients presenting with an existing such disorder. Typically, the subject has been diagnosed as having the disorder for which treatment is sought. Further, subjects can be monitored during the course of treatment for any change in the disorder (*e.g.*, for an increase or decrease in clinical symptoms of the disorder). Also, in some variations, the subject does not suffer from another disorder requiring treatment that involves targeting 5T4-expressing cells.

[00251] In prophylactic applications, pharmaceutical compositions or medicants comprising a protein of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) are administered to a patient susceptible to, or otherwise at risk of, a particular disorder in an amount sufficient to eliminate or reduce the risk or delay the onset of the disorder. In therapeutic applications, compositions or medicants comprising a protein of the present disclosure are administered to a patient suspected of, or already suffering from such a disorder in an amount sufficient to cure, or at least partially

arrest, the symptoms of the disorder and its complications. An amount adequate to accomplish this is referred to as a therapeutically effective dose or amount. In both prophylactic and therapeutic regimes, agents are usually administered in several dosages until a sufficient response (*e.g.*, inhibition of inappropriate angiogenesis activity) has been achieved. Typically, the response is monitored and repeated dosages are given if the desired response starts to fade.

[00252] To identify subject patients for treatment according to the methods of the disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof), accepted screening methods can be employed to determine risk factors associated with specific disorders or to determine the status of an existing disorder identified in a subject. Such methods can include, for example, determining whether an individual has relatives who have been diagnosed with a particular disorder. Screening methods can also include, for example, conventional work-ups to determine familial status for a particular disorder known to have a heritable component. For example, various cancers are also known to have certain inheritable components. Inheritable components of cancers include, for example, mutations in multiple genes that are transforming (*e.g.*, Ras, Raf, EGFR, cMet, and others), the presence or absence of certain HLA and killer inhibitory receptor (KIR) molecules, or mechanisms by which cancer cells are able to modulate immune suppression of cells like NK cells and T-cells, either directly or indirectly (*see, e.g.*, Ljunggren and Malmberg, *Nature Rev. Immunol.* 7:329-339, 2007; Boyton and Altmann, *Clin. Exp. Immunol.* 149:1-8, 2007). Toward this end, nucleotide probes can be routinely employed to identify individuals carrying genetic markers associated with a particular disorder of interest. In addition, a wide variety of immunological methods are known in the art that are useful to identify markers for specific disorder. For example, various ELISA immunoassay methods are available and well-known in the art that employ monoclonal antibody probes to detect antigens associated with specific tumors. Screening can be implemented as indicated by known patient symptomology, age factors, related risk factors, etc. These methods allow the clinician to routinely select patients in need of the methods described herein for treatment. In accordance with these methods, targeting pathological, 5T4-expressing cells can be implemented as an independent treatment program or as a follow-up, adjunct, or coordinate treatment regimen to other treatments.

[00253] For administration, a protein of the present disclosure (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) may be formulated as a pharmaceutical composition. A pharmaceutical composition may comprise: (i) a

5T4-binding polypeptide, a 4-1BB-binding polypeptide, and/or multispecific binding protein thereof (*e.g.*, an anti-5T4 x anti-4-1BB molecule); and (ii) a pharmaceutically acceptable carrier, diluent or excipient. A pharmaceutical composition comprising a 5T4-binding polypeptide, a 4-1BB-binding polypeptide, and/or multispecific binding protein thereof (*e.g.*, an anti-5T4 x anti-4-1BB molecule) can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic molecule is combined in a mixture with a pharmaceutically acceptable carrier, diluent or excipient. A carrier is said to be a “pharmaceutically acceptable carrier” if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers, diluents or excipients are well-known to those in the art. (*See, e.g.*, Gennaro (ed.), *Remington's Pharmaceutical Sciences* (Mack Publishing Company, 19th ed. 1995).) Formulations can further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, *etc.* In certain embodiments, a pharmaceutical composition comprises a 5T4-binding polypeptide, a 4-1BB-binding polypeptide, and/or multispecific binding protein thereof (*e.g.*, an anti-5T4 x anti-4-1BB molecule) that is a homodimer or a heterodimer. A “homodimer” may be a dimer formed from two identical polypeptides (*e.g.*, an anti-5T4 x anti-4-1BB molecule as described herein).

[00254] A pharmaceutical composition comprising a polypeptide or protein described herein may be formulated in a dosage form selected from the group consisting of: an oral unit dosage form, an intravenous unit dosage form, an intranasal unit dosage form, a suppository unit dosage form, an intradermal unit dosage form, an intramuscular unit dosage form, an intraperitoneal unit dosage form, a subcutaneous unit dosage form, an epidural unit dosage form, a sublingual unit dosage form, and an intracerebral unit dosage form. The oral unit dosage form may be selected from the group consisting of: tablets, pills, pellets, capsules, powders, lozenges, granules, solutions, suspensions, emulsions, syrups, elixirs, sustained-release formulations, aerosols, and sprays.

[00255] A pharmaceutical composition comprising polypeptide or protein described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) may be administered to a subject in a therapeutically effective amount. According to the methods of the present disclosure, polypeptide or protein described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) can be

administered to subjects by a variety of administration modes, including, for example, by intramuscular, subcutaneous, intravenous, intra-atrial, intra-articular, parenteral, intranasal, intrapulmonary, transdermal, intrapleural, intrathecal, and oral routes of administration. For prevention and treatment purposes, an agonist (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) can be administered to a subject in a single bolus delivery, via continuous delivery (*e.g.*, continuous transdermal delivery) over an extended time period, or in a repeated administration protocol (*e.g.*, on an hourly, daily, weekly, or monthly basis).

[00256] Determination of effective dosages in this context is typically based on animal model studies followed up by human clinical trials and is guided by determining effective dosages and administration protocols that significantly reduce the occurrence or severity of the subject disorder in model subjects. Effective doses of the compositions of the present disclosure vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, whether treatment is prophylactic or therapeutic, as well as the specific activity of the composition itself and its ability to elicit the desired response in the individual. Usually, the patient is a human, but in some diseases, the patient can be a nonhuman mammal. Typically, dosage regimens are adjusted to provide an optimum therapeutic response, *i.e.*, to optimize safety and efficacy. Accordingly, a therapeutically effective amount is also one in which any undesired collateral effects are outweighed by the beneficial effects of administering a 5T4-binding polypeptide, a 4-1BB-binding polypeptide, and/or a multispecific binding protein thereof (*e.g.*, an anti-5T4 x anti-4-1BB molecule) as described herein. For administration of a protein or polypeptide described herein, a dosage may range from about 0.1 µg to 100 mg/kg or 1 µg/kg to about 50 mg/kg, and more usually 10 µg to 5 mg/kg of the subject's body weight. In more specific embodiments, an effective amount of the agent is between about 1 µg/kg and about 20 mg/kg, between about 10 µg/kg and about 10 mg/kg, or between about 0.1 mg/kg and about 5 mg/kg. Dosages within this range can be achieved by single or multiple administrations, including, *e.g.*, multiple administrations per day or daily, weekly, bi-weekly, or monthly administrations. For example, in certain variations, a regimen consists of an initial administration followed by multiple, subsequent administrations at weekly or bi-weekly intervals. Another regimen consists of an initial administration followed by multiple, subsequent administrations at monthly or bi-monthly

intervals. Alternatively, administrations can be on an irregular basis as indicated by monitoring clinical symptoms of the disorder.

[00257] Dosage of the pharmaceutical composition comprising a polypeptide or protein described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) can be varied by the attending clinician to maintain a desired concentration at a target site. For example, if an intravenous mode of delivery is selected, local concentration of the agent in the bloodstream at the target tissue can be between about 0.01-50 nanomoles of the composition per liter, sometimes between about 1.0 nanomole per liter and 10, 15, or 25 nanomoles per liter depending on the subject's status and projected measured response. Higher or lower concentrations can be selected based on the mode of delivery, *e.g.*, trans-epidermal delivery versus delivery to a mucosal surface. Dosage should also be adjusted based on the release rate of the administered formulation, *e.g.*, nasal spray versus powder, sustained release oral or injected particles, transdermal formulations, *etc.* To achieve the same serum concentration level, for example, slow-release particles with a release rate of 5 nanomolar (under standard conditions) would be administered at about twice the dosage of particles with a release rate of 10 nanomolar.

[00258] The proteins and polypeptides described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof) (*e.g.*, an anti-5T4 x anti-4-1BB molecule) may also be administered at a daily dosage of from about 0.001 to about 10 milligrams (mg) per kilogram (mpk) of body weight, preferably given as a single daily dose or in divided doses about two to six times a day. For administration to a human adult patient, the therapeutically effective amount may be administered in doses in the range of 0.2 mg to 800 mg per dose, including but not limited to 0.2 mg per dose, 0.5 mg per dose, 1 mg per dose, 5 mg per dose, 10 mg per dose, 25 mg per dose, 100 mg per dose, 200 mg per dose, and 400 mg per dose, and multiple, usually consecutive daily doses may be administered in a course of treatment. The proteins and polypeptides described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof such as an anti-5T4 x anti-4-1BB molecule) can be administered at different times of the day. In one embodiment the optimal therapeutic dose can be administered in the evening. In another embodiment the optimal therapeutic dose can be administered in the morning. The total daily dosage of the proteins and polypeptides described herein thus can in one embodiment range from about 1 mg to about 2 g,

and often ranges from about 100 mg to about 1.5 g, and most often ranges from about 200 mg to about 1200 mg. In the case of a typical 70 kg adult human, the total daily dose of the anti-5T4 therapeutic can range from about 2 mg to about 1200 mg and will often range, as noted above, from about 0.2 mg to about 800 mg.

[00259] With particular regard to treatment of solid tumors, protocols for assessing endpoints and anti-tumor activity are well-known in the art. While each protocol may define tumor response assessments differently, the RECIST (Response evaluation Criteria in solid tumors) criteria is currently considered to be the recommended guidelines for assessment of tumor response by the National Cancer Institute (*see* Therasse *et al.*, *J. Natl. Cancer Inst.* 92:205-216, 2000). According to the RECIST criteria tumor response means a reduction or elimination of all measurable lesions or metastases. Disease is generally considered measurable if it comprises lesions that can be accurately measured in at least one dimension as ≥ 20 mm with conventional techniques or ≥ 10 mm with spiral CT scan with clearly defined margins by medical photograph or X-ray, computerized axial tomography (CT), magnetic resonance imaging (MRI), or clinical examination (if lesions are superficial). Non-measurable disease means the disease comprises of lesions < 20 mm with conventional techniques or < 10 mm with spiral CT scan, and truly non-measurable lesions (too small to accurately measure). Non-measurable disease includes pleural effusions, ascites, and disease documented by indirect evidence.

[00260] The criteria for objective status are required for protocols to assess solid tumor response. Representative criteria include the following: (1) Complete Response (CR), defined as complete disappearance of all measurable disease; no new lesions; no disease related symptoms; no evidence of non-measurable disease; (2) Partial Response (PR) defined as 30% decrease in the sum of the longest diameter of target lesions (3) Progressive Disease (PD), defined as 20% increase in the sum of the longest diameter of target lesions or appearance of any new lesion; (4) Stable or No Response, defined as not qualifying for CR, PR, or Progressive Disease. (*See* Therasse *et al.*, *supra.*)

[00261] Additional endpoints that are accepted within the oncology art include overall survival (OS), disease-free survival (DFS), objective response rate (ORR), time to progression (TTP), and progression-free survival (PFS) (*see Guidance for Industry: Clinical Trial Endpoints for the Approval of Cancer Drugs and Biologics*, April 2005, Center for Drug Evaluation and Research, FDA, Rockville, MD.)

[00262] Pharmaceutical compositions comprising the proteins and polypeptides described herein (*e.g.*, 5T4-binding polypeptides, 4-1BB-binding polypeptides, and/or multispecific binding proteins thereof such as an anti-5T4 x anti-4-1BB molecule) can be supplied as a kit comprising a container that comprises the pharmaceutical composition as described herein. A pharmaceutical composition can be provided, for example, in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a pharmaceutical composition. Such a kit can further comprise written information on indications and usage of the pharmaceutical composition.

[00263] The disclosure will be further clarified by the following examples, which are intended to be purely exemplary of the disclosure and in no way limiting.

EXAMPLES

Example 1: Conversion of “1618” anti-CD137 monoclonal antibody to scFv format and generation of bispecific anti-CD137 x anti-5T4 molecules in ADAPTIR™ format (scFv-Fc-scFv)

[00264] In order to generate a nucleotide sequence encoding a 1618 scFv, nucleotide sequences encoding the variable domains of the heavy chain (V_H) and light chain (V_L) (SEQ ID NO: 28 and SEQ ID NO: 16, respectively) of the 1618 anti-4-1BB monoclonal antibody were synthesized and/or amplified from existing plasmid DNA and linked together by a (Gly)₄Ser-based linker using standard molecular biology techniques those described in, but not limited to *e.g.*, PCT Application Publication Nos. WO 2007/146968, WO 2010/040105 and WO 2010/003108; U.S. Patent Application Publication Nos. 2006/0051844; and U.S. Patent No. 7,166,707. The nucleotide sequence of the 1618 scFv was then fused to a modified human IgG1 Fc region sequence comprising point mutations to eliminate the effector function activities of the Fc region. Similarly, nucleotide sequences encoding the V_H and V_L domains of the 1210 anti-5T4 monoclonal antibody (SEQ ID NOs: 38 and 68, respectively) were linked together by an additional (Gly)₄Ser-based linker. The resulting bispecific molecules were expressed via transient transfection of HEK-293 or Chinese Hamster Ovary (CHO) cells and purified from conditioned media using Protein A affinity purification (ProA) and size exclusion chromatography (SEC). Protein purity was

determined by analytical SEC after each of the Protein A and SEC purification steps. Endotoxin levels were determined by using the Endosafe PTS instrument according to the manufacturer's instructions to assure that the in vitro activity assay results would not be confounded by the presence of endotoxin. Each protein batch was buffer-exchanged into PBS as part of the SEC purification process, concentrated to 1 mg/mL, sterile-filtered, and stored at 4° C until needed. Protein concentration was determined from the absorbance at 280 nm and using the theoretical extinction coefficient.

[00265] In some instances, the bispecific molecules were comprised of 2 scFvs and 1 Fc domain (scFv-Fc-scFv) with the following structure, from N-terminus to C-terminus: 1618 scFv – Fc – 1210 scFv. Additional bispecific molecules were generated in which the 1618 scFv was placed on the C-terminus of the construct and the 1210 scFv on the N-terminus (i.e., 1210 scFv – Fc – 1618 scFv). However, molecules with this alternative orientation had less desirable properties when evaluated compared to the 1618 scFv – Fc – 1210 scFv-Fc-scFv molecules.

[00266] Most, but not all, of the 1618-Fc-1210 scFv-Fc-scFv ADAPTIR™ molecules (*e.g.*, ALG.APV-006, ALG.APV-010, ALG.APV-014, and ALG.APV-018) had significantly improved expression levels when transiently transfected into HEK-293 cells compared to the Morrison format (i.e. ALG.APV-004). Table 12 contains representative data showing the improved expression levels of the ADAPTIR™ bispecific molecules (*e.g.*, ALG.APV-006, ALG.APV-010, ALG.APV-014, and ALG.APV-018) versus the Morrison bispecific constructs (ALG.APV-004). This was an unexpected result, as the amino acid sequence of the 1618 and 1210 variable domains were not modified to obtain this improvement. These results indicate that the ADAPTIR™ bispecific molecules may be beneficial for manufacturing of therapeutic protein drugs; higher expression levels are generally considered beneficial for therapeutic protein drug manufacturing as this can provide lower cost of goods and other efficiencies in the manufacturing process, such as fewer production runs needed to meet market requirements.

[00267] In addition to the apparent improved expression in HEK-293, the aggregate levels quantified after Protein A purification of the bispecific molecule from conditioned media were significantly improved in the ADAPTIR™ bispecifics (ALG.APV-006, ALG.APV-010, ALG.APV-014 and ALG.APV-018) compared to the Morrison format bispecific (ALG.APV-004), as shown in Table 12.

Table 12. HEK-293 transient expression levels and post-Protein A purity of ADAPTIR™ bispecifics vs. Morrison bispecifics

Construct	Construct Distinction	Bispecific Format	Expression Level $\mu\text{g/mL}$	% Purity Analytical SEC
ALG.APV-004	1618 mAb-1210 V _H V _L w/ stabilizing disulfide	Morrison	13.0	81.7
ALG.APV-006	1618 V _H V _L -Fc-1210 V _H V _L	ADAPTIR™	25.5	94.4
ALG.APV-010	1618 V _H V _L -Fc-1210 V _L V _H	ADAPTIR™	55.9	94.3
ALG.APV-014	1618 V _H V _L -Fc 1210 V _H V _L w/ stabilizing disulfide	ADAPTIR™	18.9	89.2
ALG.APV-018	1618 V _H V _L -Fc 1210 V _L V _H w/ stabilizing disulfide	ADAPTIR™	25.8	89.1

[00268] The order of the V_H and V_L domains was also evaluated with both the 1210 and 1618 scFvs (V_H-V_L or V_L-V_H) and it was determined that the preferred orientation of the 1618 scFv was V_H-V_L, as the expression levels of scFv-Fc-scFv molecules were significantly reduced when the V_L-V_H orientation was utilized. The preferred orientation of the 1210 scFv was V_H-V_L, which conferred improved binding to 5T4-expressing cells and *in vitro* activity in a CD137 reporter assay when compared to bispecific molecules that were comprised of the 1210 scFv in the V_L-V_H format as shown in Table 13.

Table 13. Comparison of cell binding (EC₅₀) and *in vitro* activity in a CD137 reporter assay when the 1210 scFv is either in the V_H-V_L or V_L-V_H orientation

Construct	Construct Distinction	Affinity to Human 5T4-expressing cells, EC ₅₀ (nM), measured by flow cytometry	CD137 Reporter Assay EC ₅₀ (nM)
ALG.APV-006	1618 V _H V _L -Fc-1210 V _H V _L	15.7	0.23
ALG.APV-010	1618 V _H V _L -Fc-1210 V _L V _H	50.9	2.60

[00269] The length of the linker between the V_H and V_L domains of the 1618 and 1210 scFvs was also evaluated. Changing the linker length connecting the V_H and V_L to either a

4X or 3X repeat of the Gly4Ser linker did not appear to result in a significant difference in activity or stability (data not shown), so the 4X repeat was generally used unless otherwise denoted.

Example 2: Optimization of the 1618 anti-CD137 scFv and anti-5T4 scFv binding domains

[00270] After initial characterization of ADAPTIR™ bispecific (ALG.APV-006), it was desirable to increase 1) the binding affinities of the scFv binding domains to their respective targets; 2) the biophysical stability; and 3) the *in vitro* activity. Improvements in these parameters are expected to lead to improved cost of goods, ease of manufacturing and reduced clinical dosage amounts. For binding domain optimization, random mutagenesis phage libraries were generated for both the 1618 and 1210 scFv using error-prone PCR. Briefly, each scFv was used as template in an error-prone PCR reaction using a commercial mutagenesis kit (GeneMorph II Random Mutagenesis Kit, Agilent Technologies) following the manufacturer's protocol. The epPCR products were digested, ligated into the phagemid-scFv vector, and transformed into *E. coli* SS320/M13K07 competent cells to generate the phage libraries. Five rounds of panning were performed on each library, using biotinylated 4-1BB or 5T4 ECD as antigen (for the 1618 or 1210 binding domains, respectively). Increased stringency of panning was used for each successive round by decreasing the antigen concentration and increasing the wash times. Following the final round of panning, phage output was plated and prepared for a bulk cloning of the scFv pool into the prepared ADAPTIR™ expression vector. Approximately 400 individual colonies were picked, phagemids isolated and sequenced, and the purified DNA used in high-throughput small scale 293 transient transfections (~0.6 mL culture volume). 5-day clarified supernatants were assayed for binding by Flow Cytometry and SPR. The best performing variants were then carried forward through a battery of tests, including cell binding, *in vitro* activity and various stability assays. These bispecific ADAPTIR™ variants were produced with changes to either the 1210 or 1618 scFv, while keeping the other scFv as the unmodified parental sequence. This simplified the interpretation of the data and allowed us to assess the impact of these single changes on binding, activity and stability. Beneficial point mutations were identified, based on improvements to stability, affinity or bioactivity. These were then combined to produce an additional set of ADAPTIR DNA constructs. These constructs incorporated multiple changes in either the 1210 and/or 1618 scFv. Each construct was expressed by HEK-293 cells via transient transfection in 250 mL culture volume and purified using ProA and SEC steps. Final protein purity was verified by analytical

SEC and the endotoxin levels was determined by using the Endosafe PTS instrument according to the manufacturer's instructions to assure that the in vitro activity assay results would not be confounded by the presence of endotoxin. The resulting protein was buffer-exchanged into PBS as part of the SEC purification process, concentrated to 1 mg/mL, sterile-filtered, and stored at 4°C until needed. Protein concentration was determined from the absorbance at 280 nm and using the theoretical extinction coefficient.

Example 3. Evaluation of phage-derived optimized variants

[00271] Several beneficial amino acid changes to 1210 and 1618 scFvs were identified as part of the phage panning efforts, when compared to the parent ALG.APV-006 construct. One measure of protein colloidal stability is to examine the amount of protein that is precipitated out of solution when ammonium sulfate is added to a final concentration of ~1M. Under these conditions, the parent construct ALG.APV-006 lost nearly 89% of the protein in solution (Table 14). In comparison, the changes represented by ALG.APV-099, ALG.APV-127, ALG.APV-148 and ALG.APV-150 all show reduced protein loss under these conditions, suggesting that the individual amino acid changes made in these constructs improved the colloidal stability of the ADAPTIR™ bispecifics. Another measure of protein stability is to look at the impact of applying a shear force to a protein sample and examining that sample for loss due to precipitation or formation of soluble, aggregated forms of the bispecific. When assessed in a shear assay in which the protein solution was placed in a 96-well plate and shaken at 2,000 RPM for two hours, the ALG.APV-127 mutation significantly reduced the amount of protein loss, while the other variants had minimal effect or slightly exacerbated loss (Table 14). Another measure of thermostability can be determined by using differential scanning calorimetry (DSC) or differential scanning fluorimetry (DSF) to determine the mid-point temperature of the melting curve, otherwise known as T_m. Upon determination of the T_m of the 1618 and 1210 variants, we noted that the ALG.APV-127 and ALG.APV-150 variants increased the T_m of the 1618 scFv by three to four degrees, when compared to the ALG.APV-006 parental sequence (Table 14). An increase in the value of T_m can generally be interpreted as an improvement in a folded protein's stability, as it means that the protein is more resistant to heat-induced unfolding/denaturation. ALG.APV-148 increased the thermostability of the 1210 scFv, as the T_m was increased nearly three degrees compared to the parent 1210 sequence used in ALG.APV-006.

[00272] Various combinations of the ALG.APV-099, ALG.APV-127, ALG.APV-148, and ALG.APV-150 mutations were evaluated to see if combining mutations provided additive benefit to the biophysical stability of the ADAPTIR™ bispecific constructs. These were expressed and purified using the same method and evaluated in the same assays as the individual point mutations. The results in Table 14 show that the combination of mutations included in ALG.APV-178 and ALG.APV-179, as representative examples, resulted in stability increases that were generally equivalent or better than that obtained from individual changes.

Table 14. Biophysical assessment data for non-optimized and optimized ADAPTIR™ bispecific constructs

Construct	Modified Domain	1M Ammonium Sulfate Solubility, % Protein Loss	Shear Stress, % Protein Loss	T_m (°C) 1618 scFv	T_m (°C) 1210 scFv
ALG.APV-006	Parent Sequence of 1210 and 1618	89	64	56.1	70.5
ALG.APV-099	1210	67	80	57	70.5
ALG.APV-127	1618	55	32	60.4	71.0
ALG.APV-148	1210	24	76	56.9	73.0
ALG.APV-150	1618	56	71	58.9	70.8
ALG.APV-178	1210 and 1618	18.7	24.5	61.7	72.3
ALG.APV-179	1210 and 1618	28.9	26.5	61.7	72.3

[00273] In addition to the stability assessments that were performed, the binding affinity of ALG.APV-006 and the phage derived variants were determined by Surface Plasmon Resonance (SPR) using a Biacore T-200, using the recombinant extracellular domains of human 5T4 and human CD137. ALG.APV-127 and ALG.APV-150 modifications led to a significantly tighter binding affinity to CD137 (Table 15). Similarly, it was observed that ALG.APV-099 binds significantly more tightly than ALG.APV-006 (13 vs. 149 nM, respectively).

Table 15. Summary of binding and activity assessments for non-optimized and optimized ADAPTIR™ bispecific constructs

Construct	Modified Domain	Affinity to Human CD137 by SPR KD (nM)	Affinity to Human 5T4 by SPR KD (nM)	In vitro activity EC ₅₀ (nM)
ALG.APV-006	Parent Sequences	128	149	0.23
ALG.APV-099	1210	N/A	13	0.09
ALG.APV-127	1618	58	N/A	0.03
ALG.APV-148	1210	N/A	198	0.05
ALG.APV-150	1618	97	N/A	0.03

[00274] The binding of the ALG.APV-099, ALG.APV-127, ALG.APV-148 and ALG.APV-150 variants to CD137 and 5T4 expressed on the surface of cells was compared to the binding of ALG.APV-006. In spite of the improved affinity for soluble CD137 or 5T4 displayed by some of these mutations, no appreciable difference was observed in on-cell binding experiments (data not shown). To compare the effectiveness of the phage-display derived variants at inducing target-dependent activation of CD137, the variants were compared in a CD137 reporter assay.

[00275] CD137 reporter assay: Jurkat/CD137 transfectants carrying a luciferase reporter gene under the control of an NF-κB promoter (Promega) were cultured according to the manufacturer's protocols. Jurkat/NF-κB reporter cells were cultured with CHO-K1 cells transfected with human 5T4, at approximately 15,000 reporter cells to 30,000 target cells in 96-well plates. Concentrations of bispecific molecules with final concentration ranging from 10 nM to 0.002 nM were added. Cells were cultured in a total volume of 100 μL of RPMI 1640 media supplemented with 1% fetal bovine serum, sodium pyruvate, antibiotics and non-essential amino acids. Plates were incubated at 37°C, 5% CO₂ in humidified incubators for 5 to 6 hours. One hundred microliters of Bio-Glow buffer (Promega) was added to each well and incubated for 5 to 10 minutes before measuring fluorescence. Luminescence was measured in a MicroBeta² 2450 Microplate Counter (Perkin Elmer). Nonlinear regression analysis to determine EC₅₀ values was performed in GraphPad Prism 6® graphing and statistics software.

[00276] FIG. 1 shows that all the constructs displayed agonistic function in the presence of 5T4(+) cells; no reporter activity was observed in the absence of 5T4(+) cells (not shown). The constructs ALG.APV-099, ALG.APV-127, ALG.APV-148 and ALG.APV-150

displayed better agonist function (lower EC₅₀ values) than the original ALG.APV-006 construct. Every point in the curve represents the average of duplicate wells. The Morrison format molecule ALG.APV-004 was included for comparison. The y axis shows values in relative fluorescence units (RLU) represented as percent of maximum fluorescence.

[00277] Due to the improvements obtained with the single point mutations represented by ALG.APV-099, ALG.APV-127, ALG.APV-148 and ALG.APV-150, these were combined in various ways in order to determine if the characteristics of the individuals would have additive benefit when included in the same protein. These unique mutations were evaluated in combination to derive variants of 1618 and 1210 scFvs with significantly improved properties such as ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-191, ALG.APV-196, ALG.APV-198 and ALG.APV-199. To generate the ALG.APV-191, ALG.APV-196, ALG.APV-198 and ALG.APV-199 constructs, the single point mutations were further combined with the LO1, LO2, and LO11 mutations described in Examples 22 and 23 of PCT Application No. PCT/EP2017/059656. Further, targeted phage libraries were created after analyzing regions of the 1618 and 1210 scFvs to identify regions that may contribute to instability. However, panning these libraries did not yield scFvs with improved properties (data not shown).

Example 4. Evaluation of stabilizing disulfide bonds

[00278] Point mutations to incorporate an additional cysteine residue into the V_H and V_L domains of both the 1618 and 1210 scFvs were made. When these bispecific molecules are expressed, these cysteines react to form a disulfide bond and can act to increase the stability of the scFv and confer beneficial properties to improve the manufacturing and storage of these products. Experiments were performed showing that when optimized bispecific molecules with the stabilizing disulfide in the 1210 scFv are stored for one week at 4 or 25° C, or submitted to three freeze-thaw cycles between -80 °C and room temperature, they maintain their purity better than bispecific molecules without the disulfide. Representative data is shown in Table 16. Addition of a stabilizing disulfide bond to the 1618 scFv was also evaluated. While the disulfide appeared to confer a stability benefit, the Biacore binding data suggested that there was heterogeneity in the sample leading to atypical binding kinetics (data not shown).

Table 16. Improvement in storage and freeze-thaw stability in bispecific molecules containing a stabilizing disulfide bond in the 1210 scFv

Construct	Is stabilizing disulfide in 1210 scFv?	Decrease in % Purity Day 7 @ 4°C	Decrease in % Purity Day 7 @ 25°C	Change in % Purity after 3 Freeze-Thaw Cycles from -80°C to Room Temperature
ALG.APV-178	No	2.4	1.9	4
ALG.APV-179	No	1.5	1.7	4
ALG.APV-187	Yes	0.1	0.1	1
ALG.APV-191	Yes	0.0	0.0	2
ALG.APV-196	Yes	0.0	0.1	2
ALG.APV-198	Yes	0.1	0.1	2
ALG.APV-199	Yes	0.0	0.1	1

[00279] The improvement in bispecific molecule stability with the inclusion of a stabilizing disulfide bond in the 1210 scFv was also evident in a subsequent evaluation performed at 40° C. Two constructs, ALG.APV-209 and ALG.APV-210 differ by the addition of a stabilizing disulfide in the 1210 scFv of ALG.APV-210. After one week at this elevated temperature, ALG.APV-210 formed significantly less aggregated material (Table 17).

Table 17. Improved 40° C stability data for a bispecific molecule with a stabilizing disulfide bond in the 1210 scFv

Construct	Is stabilizing disulfide in 1210 scFv?	Decrease in % Purity Day 7 @ 40°C
ALG.APV-209	No	2.6
ALG.APV-210	Yes	0.2

[00280] While the inclusion of the disulfide bond in 1210 provided significant benefit in storage stability, the other stability parameters that were assessed did not detect a significant difference between the two constructs (Table 18). Both constructs performed significantly better in these assays as compared to the parent bispecific molecule ALG-006 (Table 14).

Table 18. Comparison of ALG.APV-209 to ALG.APV-210 stability data

Construct	Is stabilizing disulfide in 1210 scFv?	Modified Domain	1M Ammonium Sulfate Solubility, % Protein Loss	Shear Stress, % Protein Loss	Tm (°C) 1618 scFv	Tm (°C) 1210 scFv
ALG.APV-209	No	1210 and 1618	16.2	22.1	61.2	72.7
ALG.APV-210	Yes	1210 and 1618	13.1	23.1	61.5	73.2

Example 5: Evaluation of binding affinity of bispecific scFv2Fc proteins to human 5T4 and human CD137

Methods

[00281] Expression and purification of recombinant human 5T4 and human CD137 Extracellular Domains (ECDs): Using standard molecular biology techniques and starting with a vector encoding the full length sequence of the 5T4 and CD137 proteins, nucleotide primers were designed to amplify the extracellular regions of human 5T4 and human CD137. An additional peptide sequence was added to the c-terminus of ECD at the position where the native protein would be predicted to enter the membrane. This peptide contained recognition sequences that could be utilized for affinity purification purposes. The expression vectors were transiently transfected into HEK-293 cells. The recombinant ECD was purified from the conditioned media using immobilized metal affinity chromatography (IMAC) and size exclusion chromatography. Protein purity was verified using analytic SEC.

[00282] Surface Plasmon Resonance (SPR) affinity analyses of bispecific proteins to recombinant CD137 and 5T4 ecto-domain: SPR binding affinity studies of bispecific anti-5T4 x anti-CD137 proteins to recombinant monomeric 5T4 and CD137 ectodomain (ECD) were conducted at 25°C in HBS-EP⁺ buffer on a Biacore T200 system. Goat anti-human IgG F(ab')₂

fragment (Jackson ImmunoResearch) at 20 µg/mL in 10 mM sodium acetate (pH 4.5) was immobilized at a density of 2000 response units (RU) onto the flow cell of a CM5 research-grade sensor chip (GE) by standard amine coupling chemistry. Each bispecific variant at 200 nM in HBS-EP+ buffer was captured in the flow cell with the immobilized anti-human IgG at a flow rate of 50 µL/min for 60 sec to reach ~500 RU response, leaving one flow cell surface unmodified as the reference. Following a 30 sec stabilization period, four different concentrations of each ECD (0, 20, 60, and 180 nM) were injected at 50 µL/min for either 120 sec followed by a 240 sec dissociation period (for 5T4) or 90 sec followed by a 180 sec dissociation period (for 4-1BB). Regeneration was achieved by duplicate injections of 10 mM glycine (pH 1.5) at a flow rate of 50 µL/min for 30 sec followed by HBS-EP+ buffer stabilization for 1 min.

[00283] Sensorgrams obtained from kinetic SPR measurements were analyzed by the double subtraction method. The signal from the reference flow cell was subtracted from the analyte binding response obtained from flow cells with immobilized or captured ligands. Buffer reference responses were then averaged from multiple injections. The averaged buffer reference responses were then subtracted from analyte binding responses, and the final double-referenced data were analyzed with Biacore T200 Evaluation software (2.0, GE), globally fitting data to derive kinetic parameters. All sensorgrams were fitted using a simple one-to-one binding model.

Results

[00284] Table 19 shows the binding parameters determined by SPR for ADAPTIR™ bispecifics that bind to CD137 and 5T4. It should be noted that performing affinity measurements in this format, with the bispecific molecule captured on the chip and injecting recombinant monomeric CD137 or 5T4 ECD should result in true affinity values that are not confounded by avidity effects. As Table 19 illustrates, it was possible via careful screening of the randomized phage libraries to isolate binding domain variants that led to significant improvement in binding affinity either anti-CD137 and/or anti-5T4 scFv. A large reduction in the Hu CD137 KD value was achieved with the optimized ADAPTIR™ bispecifics ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-209, and ALG.APV-210 in comparison to the unoptimized parent construct ALG.APV-006. Similarly, ALG.APV-179, ALG.APV-187, ALG.APV-209, and ALG.APV-210 exhibited improved affinity values for human 5T4 when compared to the unoptimized ALG.APV-006.

Table 19. Binding affinity (K_D), disassociation, and association constants for bispecific molecules binding to recombinant human CD137 or human 5T4

Construct	Affinity to Hu CD137 by SPR			Affinity to Hu 5T4 by SPR		
	K_D (nM)	k_a (1/Ms)	k_d (1/s)	K_D (nM)	k_a (1/Ms)	k_d (1/s)
ALG.APV-006	210	2.8E+05	5.9E-02	109	2.4E+04	2.6E-03
ALG.APV-178	70	3.9E+05	2.7E-02	106	2.3E+04	2.5E-03
ALG.APV-179	71	3.9E+05	2.8E-02	29	2.2E+04	6.2E-04
ALG.APV-187	73	3.7E+05	2.7E-02	35	2.1E+04	7.2E-04
ALG.APV-209	59	5.6E+05	3.3E-02	66	1.4E+04	9.4E-04
ALG.APV-210	59	5.5E+05	3.3E-02	79	1.5E+04	1.2E-03

Example 6: Evaluation of thermal stability of bispecific scFv-Fc-scFv proteins to Human 5T4 and Human CD137

[00285] Differential Scanning Calorimetry (DSC) and Differential Scanning Fluorimetry (DSF) are tools typically employed to measure assess the structural stability of recombinant proteins. By measuring the energy required to increase the temperature of a protein sample, it is possible to determine the midpoint temperature of melting (T_m) of individual protein domains. It is generally accepted that protein domains with higher T_m values are considered to be more stable. DSF analysis was performed with a set of optimized bispecific ADAPTIR™ bispecific proteins (ALG.APV-178, ALG.APV-179 and ALG.APV-187) and compared to the unoptimized ADAPTIR™ bispecific version (ALG.APV-006) (Table 20). A significant increase in T_m of the 1618 anti-CD137 and 1210 anti-5T4 was observed after incorporation of beneficial mutations that were identified by a random mutagenesis phage display approach described above.

Table 20. Midpoint of melting temperature, T_m , of optimized ADAPTIR™ bispecifics versus unoptimized constructs

Optimized?	Construct	T_m , Anti-CD137 scFv, °C	T_m , Anti-5T4 scFv, °C
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N	ALG.APV-006	56.5	70.2
Y	ALG.APV-178	60.7	73.0
Y	ALG.APV-179	61.1	73.0
Y	ALG.APV-187	61.1	73.5

Example 7: Binding of bispecific scFv-Fc-scFv proteins molecules to human and non-human primate CD137(+) cell lines

[00286] To confirm that binding to CD137 on the surface of cells was not lost upon scFv conversion of the variable domains or the mutations introduced, flow cytometry was used to quantitate binding of constructed bi-specific CD137 x 5T4 binding ADAPTIR™ molecules to cell lines expressing human or cynomolgus macaque CD137.

[00287] Binding studies on CHO-K1 cells lines expressing CD137 were performed by standard flow cytometry-based staining procedures. CHO-K1 cells (generated by Alligator) were transfected with human or cynomolgus macaque CD137. A typical experiment would label approximately 100,000 cells per well, in 96-well plates, with a range of 100 nM to 0.05 nM binding molecule in 50 µL of saline buffer with 2% BSA and 2 mM EDTA, for 30 min on ice, followed by washes and incubation with PE-labeled secondary antibody, goat anti-human IgG Fcγ (Jackson Laboratory), for 20 minutes on ice. Signal from bound molecules was detected using a LSR-II™ or a Symphony A3 flow cytometer (BD Biosciences) and analyzed by FlowJo flow cytometry analysis software. Median fluorescence intensity (MFI-median) of bound molecules on live cells was determined after exclusion of doublets cells. Nonlinear regression analysis to determine EC₅₀ values was performed in GraphPad Prism 6® graphing and statistics software.

[00288] FIG. 2 shows the binding curves of seven ADAPTIR™ molecules (ALG.APV-006, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-191, ALG.APV-196 and ALG.APV-198) to the CHO-K1/human CD137 cell line. All the molecules showed similar levels of binding (EC₅₀ values) and saturation. FIG. 3 shows the binding curves of the same seven constructs to the CHO-K1/cynomolgus CD137 cell line. Similar levels of binding and saturation were observed by all seven constructs on the cynomolgus target. FIG. 12 shows the binding curves of six ADAPTIR™ molecules (ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-208, ALG.APV-209, and ALG.APV-210) and the Morrison format control ALG.APV-004 to the CHO-K1/human CD137 cell line. All the molecules bound with similar EC₅₀ values in the range of 0.3 to 0.6 nM, and similar levels of saturation.

Example 8: Binding of bispecific scFv-Fc-scFv proteins to human and non-human primate 5T4(+) cell lines

[00289] To compare the binding of the 5T4 on the surface of cancer cells after changes to the scFv domains were introduced, flow cytometry was used to quantitate binding of constructed bi-specific CD137 x 5T4 binding ADAPTIR™ molecules to cell lines expressing 5T4.

[00290] Binding of bispecific CD137 x 5T4 molecules to 5T4(+) cell lines: Binding characteristics of the bispecific ADAPTIR™ molecules (ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-196, ALG.APV-208, ALG.APV-209 and ALG.APV-210) were compared. Binding studies were performed by standard flow cytometry-based staining procedures two cell lines expressing 5T4: CHO-K1 cells (obtained from Alligator) transduced with human or cynomolgus macaque 5T4 and SKOV-3 human ovarian adenocarcinoma cells which express (human) 5T4. All labeling and washes were performed in U-bottom 96-well plates in saline buffer with 0.1 % BSA and 2 mM EDTA. Cells were plated at approximately 100,000 cells per well and incubated with a range of 100 nM to 0.05 nM concentrations of test molecules in 50 µL volume/well, for 30 minutes on ice. Cells were washed three times then incubated for another 30 min on ice with fluorescently-labeled secondary polyclonal antibody, F(ab')₂ goat anti-human IgG (Jackson ImmunoResearch Laboratories). The cells were then washed twice and the samples acquired in a BD LSRII or a Symphony A3 flow cytometer. The sample files were analyzed using FlowJo software; the mean fluorescence intensity (MFI-mean) or median fluorescence intensity (MFI-median) of the live population of cells in each well was calculated after gating on live cells (forward vs side scatter). Nonlinear regression analysis to determine EC₅₀ values was performed in GraphPad Prism 6® graphing and statistics software.

[00291] FIG. 4 shows the binding curves of seven ADAPTIR™ molecules (ALG.APV-006, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-191, ALG.APV-196, and ALG.APV-198) to the CHO-K1/human 5T4 cell line. The construct ALG.APV-006 bound with an EC₅₀ value >10 nM, while the remaining ADAPTIR™ constructs (ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-191, ALG.APV-196, and ALG.APV-198) bound with EC₅₀ values in the range of 3 to 10 nM. The differences in EC₅₀ values between constructs were more evident when binding assays were conducted on SKOV-3 cells. SKOV-3 cells express approximately 10-fold lower levels of human 5T4 than the CHO-K1/human 5T4 transfectants

(FIG. 5). FIG. 6 shows the binding curves of the ALG.APV-006, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-196, and ALG.APV-198 of constructs on SKOV-3 cells. The constructs ALG.APV-006, ALG.APV-078, ALG.APV-179, and ALG.APV-187 bound with slightly lower affinity (higher EC₅₀ values) and higher maximum binding levels than the ALG.APV-196 and ALG.APV-198 constructs. FIG. 7 shows the binding curves of ALG.APV-006, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-191, ALG.APV-196, and ALG.APV-198 on the CHO-K1/cynomolgus 5T4 transfectants. Similar to the binding profile on the human 5T4(+) cells, constructs ALG.APV-006, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-191, ALG.APV-196, and ALG.APV-198 displayed lower affinity and higher maximum binding levels than constructs ALG.APV-196 and ALG.APV-198.

[00292] FIG. 13 shows the binding curves of six ADAPTIR™ molecules (ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-208, ALG.APV-209 and ALG.APV-210) and the Morrison format control ALG.APV-004 to murine CT26 cells (ATCC) expressing human 5T4. These cells express levels of human 5T4 comparable to those observed on SKOV-3 cells (FIG. 5). All the constructs bound with EC₅₀ values in the range of 1 to 3 nM. Constructs ALG.APV-179, ALG.APV-187, ALG.APV-209, and ALG.APV-210 showed higher levels of maximum binding than ALG.APV-178 and ALG.APV-208. The Morrison format control molecule ALG.APV-004 showed the lowest levels of maximum binding. FIG. 14 shows the binding curves of ALG.APV-004, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-208, ALG.APV-209, and ALG.APV-210 on the CHO-K1/cynomolgus 5T4 transfectants. All the molecules bound with similar EC₅₀ values, in the range of 6 to 10 nM, and similar levels of saturation. FIG. 15 shows the binding curves of ALG.APV-004, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-208, ALG.APV-209 and ALG.APV-210 on a human cell line expressing neither CD137 nor 5T4 (MOLM13 cells, ATCC). None of the molecules showed binding at any concentration.

Example 9: Binding of a 5T4-CD137 bispecific antibody to 5T4 expressing cell lines

[00293] Experiments were performed to determine the binding of ALG.APV-210 to human 5T4 expressed on cells at different receptor densities. Both human tumor cell lines with endogenous levels of 5T4 and cells transfected to produce 5T4 were assessed for target binding of ALG.APV-210.

Materials and Methods

[00294] Biotinylation: In order to detect ALG.APV-210 binding to 5T4-expressing cell lines, ALG.APV-210 was first biotinylated. The quality of the biotinylated protein and the impact on binding to its targets was assessed using ELISA.

[00295] FACS staining and analysis of 5T4-expressing cell lines: Prior to the FACS staining, all cell lines were characterized for their 5T4 expression levels using a receptor density kit (Quantum Simply Cellular, anti-human IgG, Bangs Laboratories, Inc). Information on the cell lines used and their respective 5T4 expression densities are shown in Table 21. Adherent cell lines were cultured according to their supplier's instructions and were harvested from their cell culture flasks using Trypsin/EDTA, counted in R10 medium in a Burkner chamber, and diluted in FACS buffer (PBS + 0.5% BSA, 0.05% NaN₃) to a concentration of 4×10^6 cells/mL. 50 μ L (0.20×10^6) of cell solution was added to a 96-well FACS staining plate (Falcon # 351190). Cells were incubated for 20 min in 4° C with 50 μ L of the biotinylated ALG.APV-210 (2x final concentration). The antibodies were added in a serial dilution starting at 20 μ g/mL titrating down in a 12 step 1/2 dilution down to 0.010 μ g/mL. Following 2 x washing with FACS buffer, cells were incubated for 30 min in 4° C with 100 μ L of a secondary antibody (streptavidin-PE, BD #554061, diluted 1/500). Following 2 x washing steps with FACS buffer, cells were re-suspended in 200 μ L of cell fixation (BD CellFIX 10x BD, #340181, diluted in MQ H₂O). Cells were analysed for ALG.APV-210 binding using flow cytometry (FACSVerse) by gating on single cells. MFI values (secondary antibody only subtracted) from 1 out of 2 independent experiments, as well as normalized data and EC₅₀-values of pooled data from 2 experiments were determined and plotted using GraphPad Prism 7, non-linear regression log (agonist) vs. normalized response – variable slope.

Table 21: Cell lines expressing 5T4 and receptor densities/cell

Cells	Cancer type	5T4 density/cell	EC ₅₀ (nM)	95% CI
HCT-116	Human colon carcinoma	62,000	16	15-17
HT-29	Human colon carcinoma	30,000	12	11-14
JAR	Human choriocarcinomas	69,000	9	5-17

Cells	Cancer type	5T4 density/cell	EC ₅₀ (nM)	95% CI
JEG	Human choriocarcinomas	66,000	8	5-12
SKOV-3	Human ovarian cancer	60,000	15	12-18
BxPC-3	Pancreas cancer	150,000	20	18-22
B16-hu5T4	Transfected mouse melanoma	320,000	14	9-19
CHO-hu5T4	Transfected	4,100,000	23	18-28
CT26-hu5T4 ^{high}	Transfected mouse colon carcinoma	1,000,000	20	16-27
CT26-hu5T4 ^{int}	Transfected mouse colon carcinoma	200,000	12	11-13
CT26-hu5T4 ^{low}	Transfected mouse colon carcinoma	50,000	11	8-15

Results and Conclusions

[00296] Using ELISA, the binding of biotinylated ALG.APV-210 to 5T4 was determined to be similar to that of the non-biotinylated ALG.APV-210 (data not shown).

[00297] The binding (EC₅₀) of the biotinylated ALG.APV-210 to 5T4 when expressed on cells, either at endogenous levels on human tumor cells or on cells transfected to express 5T4 was assessed. As shown in Fig. 39 using MFI, ALG.APV-210 binds to both human tumor cells expressing endogenous levels of 5T4 and transfected cell lines in a dose-dependent manner.

[00298] Table 21 above shows the binding EC₅₀ of ALG.APV-210 to 5T4 positive human tumor cell line and to 5T4 transfected cells determined from normalized MFI values (background subtracted) from 2 pooled experiments plotted in a dose-response curve shown in Fig. 40. The mean EC₅₀ values ranged between 8-23 nM for the different 5T4 positive cell lines. In conclusion, ALG.APV-210 binds to 5T4-expressing cells at an EC₅₀ ranging between 8-23 nM.

Example 10: Agonistic function of bispecific scFv-Fc-scFv proteins in reporter assays

[00299] To compare the effectiveness of different bispecific CD137-binding molecules at inducing target-dependent activation of CD137, seven different bispecific anti-CD137 x anti-5T4 molecules were compared in a CD137 reporter assay, as described in Example 3.

CD137 reporter assay

[00300] Jurkat/CD137 transfectants carrying a luciferase reporter gene under the control of an NF- κ B promoter (Promega) were cultured according to the manufacturer's protocols. Jurkat/NF- κ B reporter cells were cultured with human primary ductal breast carcinoma cells HCC1143 (ATCC), which express human 5T4, at approximately 15,000 reporter cells to 30,000 target cells in 96-well plates. Concentrations of bispecific molecules with final concentration ranging from 10 nM to 0.002 nM were added. Cells were cultured in a total volume of 100 μ L of RPMI 1640 media supplemented with 10% fetal bovine serum, sodium pyruvate, antibiotics and non-essential amino acids. Plates were incubated at 37°C, 5% CO₂ in humidified incubators for 5 to 6 hours. One hundred microliters of Bio-Glow buffer (Promega) was added to each well and incubated for 5 to 10 minutes before measuring fluorescence. Luminescence was measured in a MicroBeta² 2450 Microplate Counter (Perkin Elmer). Nonlinear regression analysis to determine EC₅₀ values was performed in GraphPad Prism 6® graphing and statistics software.

Results

[00301] In FIG. 8, all the constructs display agonistic function in the presence of 5T4(+) cells; no reporter activity was observed in the absence of 5T4(+) cells (data not shown). The constructs ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-191, ALG.APV-196 and ALG.APV-198 displayed better agonist function (up to 6-fold lower EC₅₀ values) than the non-optimized ADAPTIRTM construct, ALG.APV-006, and the Morrison format control, ALG.APV-004. Every point in the curve represents the average of duplicate wells. The y-axis shows values in relative fluorescence units (RLU). FIG. 16 shows the activity of the bispecific constructs in Jurkat/NF- κ B reporter cells, after 5 hours of incubation in the presence of 5T4(+) cells (HCC1143) or 5T4(-) cells (MOLM13). The constructs ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-208, ALG.APV-209 and ALG.APV-210 displayed better agonist function (10-fold lower EC₅₀ values) than the Morrison format control, ALG.APV-004. No reporter activity was observed in the presence of 5T4(-) cells (MOLM13). Every point in the curve represents the average of duplicate wells. The y-axis shows values in relative fluorescence units (RLU).

Example 11: Agonistic function of bispecific scFv-Fc-scFv proteins in primary T cell assays

[00302] The agonistic function of different anti-CD137 x anti-5T4 bispecific molecules at inducing target-dependent activation of CD137 was tested in cultures of peripheral blood mononuclear cells (PBMC). In order to test the function of the anti-CD137 bispecific constructs, primary PMBC were stimulated with anti-CD3 antibodies to upregulate CD137, which is not expressed on resting T cells. Co-stimulation of primary T cells through the TCR and CD137 enhances T-cell secretion of the cytokine interferon gamma (IFN- γ).

CD137 stimulation of PBMC:

[00303] Peripheral blood mononuclear cells (PBMC) were isolated from human blood using standard ficoll gradients. The isolated cells were washed in saline buffer. PBMC were cultured with CHO-K1/human 5T4 cells, at approximately 120,000 PBMC to 30,000 5T4 (+) cells in 96-well plates. Anti-CD3 antibody OKT3 (eBioscience) was added to all the wells at a concentration of 0.1 μ g/mL. Cells were cultured in a total volume of 200 μ l of RPMI 1640 media supplemented with 1 % fetal bovine serum, sodium pyruvate, antibiotics and non-essential amino acids. Plates were incubated at 37°C, 5% CO₂ in humidified incubators for 72 hours. One hundred microliters of media were collected at 72 hours. Levels of the IFN- γ were measured in the supernatant using Milliplex® kits (Millipore) using the manufacturer's instructions. Data was collected in a Bio-Plex Reader 200 System (Bio-Rad). Nonlinear regression analysis to determine EC₅₀ values was performed in GraphPad Prism 6® graphing and statistics software.

[00304] FIG. 9 shows the levels of IFN- γ induced by ALG.APV-006, ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-196, ALG.APV-198 and the Morrison format control ALG.APV-004. All the constructs induced higher levels of IFN- γ than anti-CD3 alone, in a dose-dependent manner. Every point in the curve represents the average of duplicate wells. FIG. 17 shows the levels of IFN- γ induced in primary PBMC cultures at 72 by ALG.APV-178, ALG.APV-179, ALG.APV-187, ALG.APV-208, ALG.APV-209 and ALG.APV-210 and the Morrison format control construct ALG.APV-004 in the presence of CHO-K1/human 5T4 cells. All the constructs induced higher levels of IFN- γ than anti-CD3 alone, in a dose-dependent manner. Every point in the curve represents the average of duplicate wells.

[00305] The functional activity of anti-5T4 x anti-CD137 bispecific constructs was also evaluated in a CD8+ T cell assay wherein cells were cultured in microtiter plates coated with a 5T4-Fc construct and an anti-CD3 antibody.

5T4 stimulation of purified T cells:

[00306] PBMCs were isolated by density gradient centrifugation using Ficoll-Paque (ρ 1.077 g/mL) (GE Healthcare #17-1440-02) from leukocyte concentrates obtained from healthy donors (Clinical Immunology and Transfusion Medicine, Labmedicin Region Skåne, Lund Sweden). CD8+ T cells were enriched by negative selection using the CD8+ T cell isolation kit (Miltenyi 130-096-495). Plates were coated overnight at 4° C with 3 μ g/mL α -CD3, clone OKT3 (Affymetrix eBioscience #16-0037-85), washed and coated with 5 μ g/mL 5T4-Fc for 2 h at 37° C. After coating with 5T4-Fc, plates were washed and blocked for a minimum of 30 minutes with RPMI (Gibco # 61870010) containing 10% FCS (Heat inactivated, Gibco # 10270-106 lot 41Q9248K) and 10 mM Hepes (Gibco # 15630056). Bispecific constructs were diluted in RPMI containing 10% FCS and 10 mM Hepes and added to the plates 30 minutes before addition of CD8+ T cells (0.07×10^6 cells/well). Assay plates were incubated for 72 h at 37°C, after which culture supernatants were harvested. IFN- γ levels in the supernatants were measured by ELISA (BD OptiEIA #555142). The experiment was repeated twice with a total of 3 donors. Results from the first experiment are shown in FIG. 11A. Results of control samples, wherein cells and bispecific constructs were cultured in plates that were not coated with 5T4-Fc, are shown in FIG. 11B.

[00307] FIG. 11A shows that the ADAPTIRTM format anti-5T4 x anti-CD137 bispecific constructs (ALG.APV-178, ALG.APV-179, ALG.APV-196, and ALG.APV-198) have increased functional activity compared to the Morrison format bispecific constructs (ALG.APV-004), demonstrated by an increase in the IFN- γ levels present in the culture supernatants in the presence of 5T4. In fact, the ADAPTIRTM format constructs induced IFN- γ levels that were 2 – 4.5 times higher than those induced by the Morrison format constructs (FIG. 11A).

Example 12: Antigen dependent localization of bispecific antibodies in 5T4 positive tumors *in vivo*

[00308] **Antigen-dependent localization of the bispecific constructs targeting 5T4 and 4-1BB to 5T4-positive B16-5T4 tumors** was evaluated in wild type C57BL/6 mice.

Materials and Methods

[00309] Mice: 8 week old, female C57BL/6 mice from Janvier, France were used for the experiments. All experiments were done by approval of the Malmö/Lund ethical committee.

[00310] Bispecific constructs: Two optimized 5T4- and CD137-targeting bispecific constructs in the ADAPTIR™ format, ALG.APV-209 and ALG.APV-210, were used. ALG.APV-004, a bispecific construct in the Morrison format, served as a positive control. Vehicle administration served as a negative control.

[00311] Cells: B16.F10 WT (B16) cells were obtained from ATCC and cultivated according to their recommendations. B16 cells expressing human 5T4 (hereafter B16-5T4) were obtained from Professor Peter Stern and grown under 1.2 mg/mL of G418 selection medium.

[00312] Methods: For the antigen dependent localization of bispecific constructs, a B16 melanoma twin tumor model was used, where each mouse received one 5T4 negative and one 5T4 positive tumor at each side of the hind flank/back. The tumor cell lines, growing in log phase, were injected subcutaneously (1×10^5 cells in 100 μ L) on day 0. Intraperitoneal construct treatments (100 μ g) were given on day 6 and 13 and mice were sacrificed on day 14 (24 h after the final treatment).

[00313] FACS Analysis: Tumors for flow cytometry analysis were mechanically and enzymatically digested using Liberase TL and DNase I (Roche) and passed through a 70 μ m strainer. The resulting single cell suspension was stained for FACS analysis. Briefly, non-specific binding of the constructs was blocked using mouse IgG or Fc block. Dead cells were excluded using Fixable Viability Stain 450 (Molecular probes) according to manufacturer's instructions. Binding of the two ADAPTIR™ bispecific constructs or the control constructs to the tumor cells was analysed using a secondary antibody: goat-anti-human Fc-PE (Jackson ImmunoResearch). Alternatively, detection of the bispecific constructs was performed using biotinylated 4-1BB antigen followed by streptavidin-PE/PerCP-Cy5.5. Samples were analyzed on a BD FACSVerse

and data was analysed using FlowJo software. Statistical analysis was performed using Mann-Whitney non-parametric T-test, 2-tail and GraphPad prism program.

[00314] Immunohistochemistry: Tumors for immunohistochemistry were snap frozen in isopropanol on dry ice. Cryosections (8 mm) were stained for 5T4 expression using a rabbit anti-human 5T4 (Abcam) or rabbit anti-human Fc (Jackson Immunoresearch) followed by anti-rabbit Brightvision – HRP (Immunologic) and DAPI staining. The immunohistochemical staining was assessed as follows; negative (0), weak staining (1+), moderate staining (2+), or strong staining (3+).

Results

[00315] Both bispecific constructs in the ADAPTIR™ format, ALG.APV-209 and ALG.APV-210, and the positive control, ALG.APV-004, localized to 5T4-expressing B16 tumors, but not to 5T4-negative tumors (B16.F10). This was demonstrated both by flow cytometry (FIG. 18A and 18B) and immunohistochemistry (FIGs. 19A and 19B).

[00316] Both the bispecific constructs (ALG.APV-209, ALG.APV-210) and the positive control (ALG.APV-004) demonstrated statistically significant 5T4-dependent localization to antigen-positive tumors compared to the negative vehicle control as measured by flow cytometry (FIG. 18A). The binding to 5T4-expressing tumors could also be detected using biotinylated 4-1BB for detection (FIG. 18B), confirming that the bispecific molecules were intact.

[00317] The localization was further demonstrated by immunohistochemistry (FIGs. 19A and 19B and Table 22). The bispecific antibodies bound strongly to 5T4 positive tumors (B16-5T4, FIG. 19A) but not to the 5T4 negative tumors (B16.F10, FIG. 19B).

Table 22. *In vivo* localization of bispecific constructs and vehicle controls

Treatment	5T4-negative tumors	5T4-positive tumors
ALG.APV-209	-	+++
ALG.APV-210	-	+++
ALG.APV-004	-	+++
Vehicle	-	-

Example 13: *In vitro* activity of a 5T4-CD137 bispecific antibody in an IFN γ release assay using human CD8 T cells and 5T4-CT26 tumor cells

[00318] The functional activity of the 5T4-CD137 bispecific antibody ALG.APV-210 was evaluated in a CD8 T cell assay, where CD8 T cells, stimulated with CD3 antibodies coated on beads, were co-cultured in plates with CT26 tumor cells expressing different levels of 5T4.

[00319] CT26 tumor cells, a murine colon carcinoma cell line (ATCC® CRL-2638) was previously transfected to express human 5T4 and then single cell sorted using flow cytometry to generate single cell clones expressing either high or low levels of human 5T4 (for receptor density, see Table 23). 5T4-CT26 tumor cells or non-transfected wildtype cells (CT26 wt) were UV irradiated using a UVB crosslinker (AnalytikJena, Lamp: 254nm). After irradiation of the plate, the cells were washed once in medium and then resuspended at a concentration of 4×10^6 cells/ml in new growth medium R10 (RPMI, Gibco #61870010 containing 10% heat inactivated FBS, Hyclone #SV30160 lot RB35944 and 10 mM Hepes, Gibco #15630056). 50 μ L (2×10^5 cells/well) of the UV-irradiated CT26-h5T4^{high}, CT26-h5T4^{low} or CT26 wt cells were added to TC treated assay plates (Eppendorf #0030 730.119) and incubated in 37° C overnight.

Table 23. 5T4 receptor density on human 5T4 transfected and single cell sorted CT26 cells

CT26 cell clone	5T4 receptor density/cell
CT26-h5T4 ^{high}	$1 \times 10^6 \pm 2 \times 10^5$
CT26-h5T4 ^{low}	$5 \times 10^4 \pm 1 \times 10^4$
CT26 wt	0

[00320] The following day after UV irradiation of the CT26 cells, human PBMC were isolated by density gradient centrifugation using Ficoll-Paque (GE Healthcare #17-1440-02) from leucocyte concentrates obtained from healthy donors (Clinical Immunology and Transfusion Medicine, Labmedicin Region Skåne, Lund Sweden). CD8+ cells were enriched by negative selection using the CD8+ T cell isolation kit (Miltenyi 130-096-495).

[00321] The bispecific antibody ALG.APV-210 was diluted in a serial dilution in R10 medium and 50 μ L was added to each well of the assay plates and incubated at 37 °C for 30 min, prior to the addition of 50 μ L of α CD3 beads (4×10^8 /mL of anti-CD3 antibodies clone: OKT-3, Affymetrix eBioscience #16-0037-85, coated on beads, diluted to 2×10^6 /mL) at a 1:1 CD8+T

cell to beads ratio, followed by the addition of 50 μ L of the effector cells (enriched CD8+ cells, 2×10^6 /mL or 1×10^5 /well). The assay plates were incubated for 72 h at 37° C, and culture supernatant harvested. IFN γ levels in the supernatants were measured by ELISA (BD OptiEIA #555142). EC₅₀ was determined by non-linear regression log (agonist) vs. Normalized response (variable slope) using GraphPad Prism 7. TOP value (maximal functional effect) was determined by calculating the mean of the two highest values of IFN γ production in the culture supernatant. The experiment was performed 5 times, including in total 12 donors.

[00322] The study provided the functional effect of the CD137-5T4 bispecific antibody ALG.APV-210 using human CD8 T cells (the effector cells) co-cultured in plates with CT26 cells expressing different levels of 5T4 (for crosslinking of CD137 via binding to 5T4). As shown in FIG. 20, the bispecific CD137-5T4 antibody ALG.APV-210 induces a potent T cell activation, measured by IFN γ release, in a dose-dependent manner in the presence of CT26 cells expressing either high (CT26-h5T4^{high}) or low (CT26-h5T4^{low}) levels of 5T4. IFN γ release is presented in FIG. 20 as normalized data of ALG.APV-210; mean values of 12 donors from 5 pooled experiments are shown.

[00323] Table 24 shows the EC₅₀ of ALG.APV-210 in the CD8 T cell assay in the presence of human 5T4 expressing CT26 cells. Log (agonist) vs. Normalized response (variable slope) mean values and 95% CI of 12 donors from 5 pooled experiments is shown in the Table. As shown in FIG. 21A and 21B, the maximal functional effect of ALG.APV-210 was dependent on the expression level of 5T4 on the CT26 cells, as illustrated by a slightly lower secretion of maximal IFN γ release in co-culture with CT26-h5T4^{low} cells compared to co-cultures with CT26-h5T4^{high} cells (the maximal effect of CT26-h5T4^{low} was 82% of CT26-h5T4^{high}). In the absence of 5T4-induced crosslinking using CT26-wt cells there is no or very low T cell activity (7% of maximal effect of CT26-h5T4^{high}). Max IFN γ release is presented in FIG. 21 as absolute values (ng/mL) (FIG. 21A) or normalized values relative to CT26-h5T4^{high} (FIG. 21B). Mean and SEM values of 12 donors from 5 pooled experiments are shown.

Table 24. Binding (EC₅₀) of ALG.APV-210 in the presence of human 5T4+ CT26 cells

	ALG.APV-210-h5T4^{high}	ALG.APV-210-h5T4^{low}
EC₅₀ (nM)	0.41	0.24
95% CI	0.36 to 0.46	0.20 to 0.28

Example 14: Binding of a 5T4-CD137 bispecific antibody to CD137 on human and cynomolgus primary CD3-stimulated CD8 T cells

[00324] A study was conducted to compare the relative binding of ALG.APV-210 towards human and cynomolgus CD137 expressed on activated primary CD8 T cells (gated from CD3-stimulated PBMC).

Materials and Methods

[00325] In order to detect ALG.APV-210 binding to primary human and cynomolgus cells in FACS, ALG.APV-210 was first biotinylated. The quality of the biotinylated protein was assessed using HPLC and the impact on binding to its targets was assessed using ELISA. ANC017, an antibody in the ADAPTIR format with the same framework as ALG.APV-210 (germline CDRs) was used as a negative control and was also biotinylated.

[00326] *Isolation and stimulation of human PBMC:* Human PBMC were isolated by density gradient centrifugation using Ficoll-Paque (GE Healthcare #17-1440-02) from leucocyte concentrates obtained from 3 human healthy donors (Clinical Immunology and Transfusion Medicine, Labmedicin Region Skåne, Lund Sweden). Non-tissue treated 96 well plates (Nunc #268200) were pre-coated overnight at 4° C with 10 µg/mL αCD3, clone OKT3 (Affymetrix eBioscience #16-0037-85). The following day, plates were washed in PBS, and human PBMC diluted in R10 (RPMI, Gibco # 61870010 containing 10% heat inactivated FBS, Hyclone #SV30160 lot RB35944 and 10 mM Hepes, Gibco #15630056), were added at a concentration of 0.2×10^6 cells/well and incubated in 37° C in wells with or without CD3 stimulation for 48h.

[00327] *Isolation and stimulation of cynomolgus PBMC:* Cynomolgus PBMC were isolated from 20 mL of cynomolgus monkey blood from 3 different donors, obtained from Silabe, France by density gradient centrifugation using Lympholyte-mammal (Cedarlane labs) according to manufacturer's instructions. Non-tissue treated 96 well plates were pre-coated overnight at 4° C with 3 µg/mL α-monkey CD3, clone FN-18 (Invitrogen #APS0301). The cynomolgus PBMC were then stimulated identically as the human PBMC described above

[00328] *FACS staining and analysis of CD8 T cells:* Following 48h of incubation with or without CD3, human and cynomolgus PBMC were harvested, pooled, recounted and diluted in FACS buffer (PBS + 0.5% BSA, 0.05% NaN₃) to a concentration of 5×10^6 cells/mL. 50 µL (0.25×10^6 cells) of cell solution was added to a 96-well FACS staining plate (Falcon #

351190). Following 10 min of Fc-blocking in RT using Beriglobin (hIgG, 200 µg/mL), cells were washed in FACS buffer and then incubated for 1h in 4°C with 100 µL of the biotinylated ALG.APV-210 or negative control. The antibodies were added in a serial dilution starting either at 5 µg/mL titrating down in a 12 step 1/3 dilution down to 0.0003 µg/mL (exp 1) or 1 µg/mL titrating down in a 12 step 1/2 dilution down to 0.0005 µg/mL (exp 2). Following 2 x washing with FACS buffer, cells were incubated for 30 min in 4° C with 50 µL of a secondary antibody (streptavidin-APC, BD #554067) and 50 µL of fluorescent conjugated antibodies against different T cell surface markers (CD4-FITC #550628, CD3-PECy7 #557749 and CD8-APC-H7 #5601797, BD). Following washing in PBS, cells were stained in 15 min in 4° C with 50 µL of fixable viability stain BV510 (in order to gate away non-viable cells), washed again and then re-suspended in 130 µL of cell fixation (BD CellFIX 10x BD, #340181, diluted in MQ H2O). Cells were analysed for ALG.APV-210 binding using flow cytometry by gating on viable, single cells expressing CD3 and CD8. MFI values (FMO subtracted) from 2 independent experiments as well as normalized pooled data from 2 experiments and EC₅₀-values were determined and plotted using GraphPad Prism 7, non-linear regression log (agonist) vs. normalized response – variable slope, n = 6 donors/group.

Results

[00329] In ELISA tests, the binding of biotinylated ALG.APV-210 to CD137 was similar to that of the non-biotinylated ALG.APV-210 (0.6 nM vs. 0.7 nM). The binding (EC₅₀) of the biotinylated ALG.APV-210 to its immunomodulatory target CD137 when expressed on activated primary human and cynomolgus CD8 T cells from CD3 stimulated PBMC was assessed and compared between the two species as well as to unstimulated cells. As shown using MFI of all CD3+CD8+ cells in FIGs. 22A, 22B, and 22C, and Table 25, ALG.APV-210 binds to both human and cynomolgus CD8 T cells in a dose-dependent manner, but not to unstimulated cells. Binding to the negative control, ANC107, was low/undetectable for CD3-stimulated or unstimulated human PBMC or cynomolgus PBMC cells. The maximum amount (MFI) of ALG.APV-210 bound to CD3-stimulated cynomolgus PBMC was higher in comparison to human PBMC, most likely due to a higher proportion of the CD3-stimulated cynomolgus PBMC expressing CD137, or due to a generally higher expression of CD137 on the cynomolgus CD8 T cells. It is uncertain whether the cynomolgus cells were more sensitive towards CD3 stimulation in comparison to the human cells, and therefore upregulated CD137 more, since the cells were

stimulated with different clones of anti-CD3 due to lack of cross-reactivity of the anti-CD3 reagents between species.

Table 25. ALG.APV-210 binding (EC₅₀) to human and cynomolgus CD8 T cells

ALG.APV-210 binding (nM)	Human CD8 T cells (gated from CD3 stim PBMC)	Cynomolgus CD8 T cells (gated from CD3 stim PBMC)
EC ₅₀ (nM)	0.235	0.236
95% CI (nM)	0.21 to 0.26	0.22 to 0.25

[00330] ALG.APV-210 binding was also assessed by looking at the percentage of CD8 T cells bound by ALG.APV-210. There was a larger percentage of cynomolgus CD8 T cells bound by ALG.APV-210 in comparison to the human CD8 T cells (71-96% for cynomolgus, 65-76% for human, see FIGs. 23A, 23B, and 23C). The binding EC₅₀ values were also calculated on the MFI of the CD137 positive cells, and the results was an EC₅₀ of 0.17 nM for binding to human CD137 and 0.20 nM for cynomolgus monkey CD137.

[00331] Taken together, the results of the study showed that ALG.APV-210 binds with a similar EC₅₀: 0.2 nM to CD3-stimulated primary CD8 T cells of human and cynomolgus, but not to unstimulated cells.

Example 15: *In vitro* activity of a 5T4-CD137 bispecific antibody in an IFN γ release assay using human or cynomolgus CD8 T cells in the presence of 5T4

[00332] A study was conducted to compare the functional activity and determine EC₅₀ of ALG.APV-210 in an agonist assay using either human or cynomolgus monkey CD8 T cells in the presence of plate immobilized 5T4.

Materials and Methods

[00333] CD8 T cells were suboptimally stimulated with plate immobilized CD3 antibodies and activated by crosslinking of CD137 via ALG.APV-210 binding to plate immobilized 5T4-Fc. CD8 T cell activation was measured by determining IFN γ release in the cell culture supernatant.

[00334] *Isolation and assay setup with human CD8 T cells:* Human PBMC were isolated by density gradient centrifugation using Ficoll-Paque (GE Healthcare #17-1440-02) from

leucocyte concentrates obtained from healthy donors (Clinical Immunology and Transfusion Medicine, Labmedicin Region Skåne, Lund Sweden). CD8⁺ cells were enriched by negative selection using the CD8⁺ T cell isolation kit (Miltenyi #130-096-495). Non-tissue treated 96-well assay plates were coated overnight at 4° C with 3 µg/mL anti-CD3 antibody (clone: OKT-3, Affymetrix eBioscience #16-0037-85). The following day, plates were washed in PBS and coated with 5 µg/mL 5T4-Fc for 2h at 37°C. After 5T4-Fc immobilization, assay plates were washed in PBS and blocked for a minimum of 30 minutes with R10 (RPMI, Gibco #61870010 containing 10% heat inactivated FBS, Hyclone #SV30160 lot RB35944 and 10 mM Hepes, Gibco #15630056). ALG.APV-210 were diluted in a serial dilution in R10 and 50 µL was added in triplicates to the assay plate 30 minutes prior to the addition of 50 µL of the effector cells (enriched CD8 T cells, 1.4×10^6 cells/mL or 0.07×10^5 cells/well). Assay plates were incubated for 72h at 37° C, and culture supernatant harvested. IFN γ levels in the supernatant was measured by ELISA (BD OptiEIA #555142). The experiment was performed 4 times, including in total 8 donors.

[00335] *Isolation and assay setup with cynomolgus CD8 T cells:* Cynomolgus (Macaca fascicularis, adult males, 3-15 years) whole blood obtained from Silabe, France was used. Red blood cells were removed by red blood cell lysis (BD Pharma lyse, BD Biosciences, #555899) according to the manufactures protocol. CD8⁺ cells were enriched by positive selection using the CD8 MicroBead kit (Miltenyi Biotec, #130-091-112). Plates coated overnight at 4° C with 1 µg/mL α -monkey CD3, clone FN-18 (Invitrogen, #APS0301), washed and coated with 5 µg/mL 5T4-Fc for 2h at 37° C. After 5T4-Fc coating, plates were washed and blocked for a minimum of 30 minutes with R10. ALG.APV-210 was diluted in R10 and 50 µL was added in triplicates to the plates 30 minutes before addition of 50 µL of CD8 T cells (0.07×10^6 cells/well). Assay plates were incubated for 72h at 37° C, and culture supernatant harvested. IFN γ levels in the supernatants were measured by ELISA (Monkey IFN γ ELISA development kit, MABTECH, #3421M-1H-20). The experiment was performed 3 times, including in total 8 donors. Obtained IFN γ levels from each human and cynomolgus donor were normalized and means were calculated. The mean of the normalized IFN γ levels from all 8 human or cynomolgus donors were pooled and the EC₅₀ was determined by non-linear regression (log agonist vs normalized response, variable slope) using GraphPad 7.

Results

[00336] As shown in FIG. 24 and in Table 26, in the human CD8 T cell assay, the bispecific CD137-5T4 antibody ALG.APV-210 had a high functional activity with an EC₅₀ of 0.2 nM. Normalized IFN γ levels from 4 experiments and a total of 8 donors were pooled and the EC₅₀ determined by nonlinear regression using GraphPad 7. Mean and SEM are presented in FIG. 24. Absolute mean IFN γ values from two donors from one representative experiment is shown in FIGs. 25A and 25B. The top values of the anti-CD3 reagents as well as background IFN γ levels varies from donor to donor within one experiment. Due to the observed variations between donors the data was normalized prior to the analysis of the pooled data set. CD8 T cell activation of ALG.APV-210 was assessed both in the presence and absence of 5T4-Fc to verify a 5T4 crosslinking dependency. The results showed that the bispecific CD137-5T4 antibody ALG.APV-210 induces a potent T cell activation, measured by IFN γ release, when cross-linked by 5T4-; in the absence of 5T4 there is no T cell activity (FIGs. 25A and 25B).

[00337] The functional effect of the CD137-5T4 bispecific antibody ALG.APV-210 when using cynomolgus CD8 T cells was also assessed. Based on pooled data from 3 experiments and a total of 8 donors, the EC₅₀ of ALG.APV-210 in the cynomolgus CD8 T cell assay was determined to be 0.4 nM (FIG. 26 and Table 26). Absolute mean values from 3 donors from one representative experiment is also shown in FIGs. 27A, 27B, and 27C. Similar to the human setting, the absolute values (including top values and background IFN γ levels) also vary between individual cynomolgus donors within one experiment. Therefore, the data was normalized prior to the EC₅₀ analysis of the pooled data set. CD8 T cell activation of ALG.APV-210 was assessed both in the presence and absence of 5T4-Fc, to verify a dependency of 5T4-induced crosslinking of CD137. As shown in FIGs. 27A-27C, the agonistic effect of ALG.APV-210 is 5T4-dependent in the cynomolgus CD8 T cell assay in a similar manner as observed in the human setting. Each of FIGs. 27A, 27B, and 27C show absolute mean and SEM values of IFN γ of 3 individual donors from one representative experiment. As negative control, wells without (w/o) 5T4 were included.

Table 26. Binding of ALG.APV-210 in human and cynomolgus CD8 T cell assay

ALG.APV-210 binding (nM)	Human CD8 T cells	Cynomolgus CD8 T cells
EC₅₀	0.2	0.4
95% CI	0.17 – 0.21	0.37 – 0.44
HillSlope	2.3	3.4

[00338] The study showed that the EC₅₀ of the CD137-5T4 bispecific antibody ALG.APV-210 in a functional CD8 T cell activation assay was comparable between human (0.2 nM) and cynomolgus monkey (0.4 nM). The T cell activation was mediated by crosslinking of CD137 which is dependent on 5T4 in both human and cynomolgus CD8 T cell assays.

Example 16: *In vitro* activity of a 5T4-CD137 bispecific antibody ALG.APV-210 in a T-cell IFN- γ secretion and T cell proliferation assays

[00339] The agonistic function of the 5T4-CD137 bispecific molecule ALG.APV-210 at inducing target-dependent activation of CD137 was evaluated in a T-cell IFN- γ secretion assay and a T-cell proliferation assay using unseparated peripheral blood mononuclear cells (PBMC). In order to test the function of the anti-CD137 bispecific constructs, primary PMBC were stimulated with anti-CD3 antibodies to upregulate CD137, which is not expressed, or is expressed at low levels, on resting T cells. Co-stimulation of primary T cells through the TCR and CD137 enhances T-cell secretion of the cytokine interferon gamma (IFN- γ).

Methods

IFN- γ secretion assay:

[00340] Peripheral blood mononuclear cells (PBMC) were isolated from healthy human blood using standard ficoll gradients. The isolated cells were washed in saline buffer to remove platelets. PBMC were cultured with CHO-K1 cells transfected with human 5T4 or empty vector, at approximately 120,000 PBMC to 30,000 CHO-K1 cells in 96-well plates. Anti-CD3 antibody OKT3 (eBioscience) was added to all the wells at a concentration of 1 ng/mL. Cells were cultured in a total volume of 200 μ L of RPMI 1640 media supplemented with 10% fetal bovine serum, sodium pyruvate, antibiotics and non-essential amino acids. Plates were incubated at 37° C, 5% CO₂ in humidified incubators for 72 hours. 100 μ L of media were collected after 72 hours of stimulation of PBMC cultures primed with a sub-optimal concentration of anti-CD3 antibodies to induce upregulation of CD137. Levels of the IFN- γ were measured in the supernatant using Milliplex® kits (Millipore) using the manufacturer's instructions. Data was collected in a Bio-Plex Reader 200 System (Bio-Rad). Nonlinear regression analysis to determine EC₅₀ values was performed in GraphPad Prism 6® graphing and statistics software.

T-cell proliferation assay:

[00341] PBMC were isolated from healthy human blood using standard ficoll gradients. Once isolated, the cells were washed in saline buffer to remove platelets. PBMC were labeled with CellTrace™ Violet (Thermofisher), washed, and cultured with irradiated CHO-K1 cells transfected with human 5T4 at approximately 120,000 PBMC to 30,000 CHO-K1 cells in 96-well plates. CHO-K1 cells were irradiated (x-ray Cell Rad irradiator, Faxitron Bioptics, LLC), and washed in medium before plating with PBMC. Anti-CD3 antibody OKT3 (eBioscience) was added to all the wells at a concentration of 5 ng/mL. Cells were cultured in a total volume of 200 µL of RPMI 1640 media (GIBCO) supplemented with 10% human serum (SIGMA), sodium pyruvate, antibiotics and non-essential amino acids. Plates were incubated at 37° C, 5% CO₂ in humidified incubators for 96 hours.

[00342] Supernatants were removed and cells were labeled in the same assay plates with antibodies to CD4, CD8 and CD5 in PBS buffer with 2% BSA and 2 mM EDTA, for 30 min on ice. 7AAD (SIGMA) was added to enable exclusion of dead cells in the analysis. After washing, cells were resuspended at 120 µL/well, and 70 µL/well volumes were collected from each well and analyzed in a flow cytometer (a LSR-IITM, BD Biosciences). Data analysis was performed using Flowjo software in two ways: calculating the % of CD8+ live T cell events (CD4- CD8+ CD5+, 7AAD-) that had undergone at least one cell division, or by counting the number of CD8+ live T cell events (CD4- CD8+ CD5+, 7AAD-). In both cases the analysis was performed after gating on lymphocytes using FSC versus SSC parameters. Each condition was tested in duplicates and the averages of each duplicate set were graphed using GraphPad Prism 6® graphing and statistics software.

Results and Conclusions

[00343] Results of the IFN-γ secretion experiments are shown in FIG. 32. Every data point in the graphs represents the average of duplicate wells. Addition of anti-CD3 alone, with no bispecific antibodies, induced very low levels of IFN-γ. The addition of the ALG.APV-210 bispecific molecule increased the secretion of IFN-γ in a dose-dependent manner. Baseline levels of IFN-γ induced by the anti-CD3 antibody, as well as the total amount of IFN-γ secretion induced by ALG.APV-210 varied by PBMC donor (FIG. 32A v. FIG. 32B). Enhanced secretion of IFN-γ

was observed in the presence of CHO-K1 cells expressing 5T4, but not in the presence of CHO-K1 cells transfected with the empty vector control. Therefore, the bispecific molecule ALG.APV-210 requires engagement of 5T4 to stimulate CD137 function as measured by IFN- γ secretion.

[00344] Results of the T cell proliferation experiments are shown in FIG. 33. Every data point in the graphs represents the average of duplicate wells. Polyclonal proliferation of CD8+ T cells was evaluated after 96 hours of stimulation with priming at a sub-optimal concentration of anti-CD3 antibody. Addition of anti-CD3 alone, with no bispecific antibody, induced low levels of CD8+ T-cell proliferation. The addition of ALG.APV-210 increased the proliferation of CD8+ T cells in a dose-dependent manner (FIG. 33A and FIG. 33B). Both the percentages of cells undergoing cell division (top panels) and the total cell counts (bottom panels) collected per well were consistent with robust cell division and accumulation of divided CD8+ T cells in the wells. The results were consistent across multiple donors.

Example 17: Functional reporter activity of ALG.APV-210 in the presence of various human tumor lines expressing a range of 5T4 protein densities

[00345] Experiments were conducted to determine whether the ALG.APV-210 construct has binding and functional reporter activity across multiple human tumor cell lines expressing 5T4 at a range of densities on the cell surface. The maximal binding capacity of ALG.APV-210 will vary depending on the cells' expression level of 5T4 tumor antigen. Binding of ALG.APV-210 was therefore tested on 3 tumor cells lines (MDA-MB-231, H1975, and TF-1) naturally expressing a range of 5T4 densities, as well as CHO-K1 cells stably transfected with 5T4 (CHO/5T4).

Material and methods

[00346] 5T4 binding assay: A 3-fold titration of ALG.APV-210 construct (ranging from 100 nM to 0.14 nM) was incubated with the human 5T4-expressing cell lines for 40 minutes in FACS buffer at 4° C, washed 3 times, and detected with PE-labeled, goat-anti-human Fc secondary antibody (Jackson ImmunoResearch). Binding of ALG.APV-210 was detected by flow cytometry. All samples were run in duplicates. Nonlinear regression analysis to determine EC₅₀ values was performed in GraphPad Prism 6® graphing and statistics software.

[00347] 5T4 protein density: The surface protein density of 5T4 present on each cell line was examined using Quantibrite™ Beads (BD Pharmingen). Quantibrite™ Beads were acquired using the exact cytometer settings used to acquire the cells for the binding curves with ALG.APV-210. The 4 bead populations were gated and the geometric mean fluorescence was determined for each peak. A linear regression Log_{10} of PE molecules per bead was plotted against the Log_{10} fluorescence using the equation $y = mx + c$, where y equals Log_{10} fluorescence and x equals Log_{10} PE molecules per bead. From that, the number of PE molecules (5T4) per cell could be calculated. For this particular assay the equation was $y = 1.036x - 1.478$. All fluorescence mean values were calculated using the geometric mean of the samples, as suggested by the manufacturer's protocol.

[00348] CD137 reporter assay: Jurkat/CD137 transfectants carrying a luciferase reporter gene under the control of an NF- κ B promoter (Promega) were cultured with H1975, TF-1, MDA-MB-231, or CHO/5T4 tumor cells at a ratio of 30,000 reporter cells to 60,000 target cells in 96-well plates. Five-fold concentrations of the ALG.APV-210 molecule, with final concentration ranging from 10 nM to 0.0006 nM, were added. Cells were cultured in a total volume of 100 μ L of RPMI 1640 media supplemented with 1% fetal bovine serum, sodium pyruvate, antibiotics and non-essential amino acids. Plates were incubated at 37° C, 5% CO₂ in humidified incubators for 5 hours. 100 μ L of Bio-Glow buffer (Promega) was added to each well, mixed, and incubated for 10 minutes before measuring luminescence. Luminescence was measured in a MicroBeta² 2450 Microplate Counter (Perkin Elmer). All data points represent duplicate samples. Nonlinear regression analysis to determine EC₅₀ values was performed in GraphPad Prism 6® graphing and statistics software. Every point in the curve represents the average of duplicate wells. The y-axis shows values in relative fluorescence units (RLU).

Results and Conclusions

[00349] Each of the tumor cell lines tested varied in the amount of 5T4 protein being expressed on the cell surface as determined using Quantibrite™ Beads. Thus, the ability of ALG.APV-210 to bind and be functionally active with a range of 5T4 expression could be examined.

[00350] ALG.APV-210 bound to a range of 5T4 cell surface-expressing tumor cell lines. As shown in FIG. 34, CHO/5T4 have 10-fold higher 5T4 expression than the endogenously

expressing 5T4 tumor cell lines. Of the three non-transfected tumor cell lines, H1975 had the highest 5T4 expression, whereas TF-1 cells had the lowest 5T4 protein density. MDA-MB-231 cells showed intermediate 5T4 expression. Surface 5T4 protein densities were calculated from the binding curves and show a range of molecules/cell from 17,000 to over 250,000. Table 27 shows 5T4 cell surface protein densities and binding EC₅₀ values generated from the binding curves. Density of 5T4 expression on tumor cell lines was calculated using a fluorescence standard.

Table 27: Summary of 5T4 densities and EC₅₀ ALG.APV-210

Tumor line	5T4 molecules/cell	EC₅₀ (nM)
CHO/5T4	278000	10
H1975	43000	20
MDA.MB-231	33000	21
TF-1	18000	71

[00351] FIG. 35 shows the results of the functional CD137 reporter assay. As shown, ALG.APV-210 was sufficiently able to induce NF-κB signaling when cross-linking CD137 via binding to CHO/hu5T4, H1975, MDA.MB.231 or TF-1 tumor lines, despite the range of cell surface 5T4 expression from 17,000 to over 250,000. Surface 5T4 protein density had an impact on maximum functional activity (Max RLU), with CHO/hu5T4 cells showing the highest RLU values. However, the three tumor cell lines induced robust reporter function. The results indicate that ALG.APV-210 functions as an agonist in the presence of 5T4-positive cells expressing a range of 5T4 densities. Table 28 shows the EC₅₀ and Max RLU induced with ALG.APV-210 in the CD137 reporter assay in the presence of the various human 5T4-expressing tumor lines.

Table 28: Summary of ALG.APV-210 EC₅₀ of and Max RLU

Tumor line	EC₅₀ (nM)	Max RLU
CHO/5T4	0.0092	3092200
H1975	0.037	2743132
MDA.MB-231	0.054	2243297
TF-1	0.063	2751522

Example 18: *In vitro* activity of a 5T4-CD137 bispecific antibody in an IFN γ release assay using human CD8 T cells and human HCT116 tumor cells

[00352] The functional activity of the 5T4-CD137 bispecific antibody ALG.APV-210 was evaluated in a CD8 T cell assay, where CD8⁺ T cells stimulated with α CD3 antibodies coated on beads were co-cultured in plates with human HCT116 tumor cells expressing endogenous levels of 5T4.

Materials and Methods

[00353] *Mitomycin C treatment of HCT116 cells:* HCT116 tumor cells, a human colon carcinoma cell line (ATCC® CCL-247™) expressing endogenous levels of human 5T4 (receptor density = 6.2×10^4 /cell, Table 21) were pre-treated with Mitomycin C (0.5 mg/mL, Sigma-Aldrich) at a concentration of 50 μ g/mL to hamper tumor cell overgrowth. After 45 min of incubation with Mitomycin C in 37° C, cells were washed three times in R10 medium (RPMI, Gibco #61870010 containing 10% heat inactivated FBS, Hyclone #SV30160 lot RB35944 and 10 mM Hepes, Gibco # 15630056) and then resuspended at a concentration of 10×10^6 cells/mL in new R10 medium. 50 μ L (5×10^5 cells/well) of the Mitomycin C treated HCT116 cells were added to TC treated assay plates (Eppendorf #0030 730.119) and incubated in 37° C overnight.

[00354] *CD8 T cell assay setup:* The following day, human PBMC were isolated by density gradient centrifugation using Ficoll-Paque (GE Healthcare #17-1440-02) from leucocyte concentrates obtained from healthy donors (Clinical Immunology and Transfusion Medicine, Labmedicin Region Skåne, Lund Sweden). CD8⁺ cells were enriched by negative selection using the CD8⁺ T cell isolation kit (Miltenyi 130-096-495). The bispecific antibody, ALG.APV-210, or an isotype control in the Adaptir format, ANC017 (variable heavy and light chain sequences for ANC017 are shown below in Tables 29A and 29B) was diluted in a serial dilution in R10 medium and 50 μ L was added to each well of the assay plates and incubated at 37° C for 30 min, prior to the addition of 50 μ L of α CD3 beads (4×10^8 /mL of anti-CD3 antibodies clone: OKT-3, Affymetrix eBioscience #16-0037-85, coated on beads, diluted to 2×10^6 /mL) at a 1:1 CD8⁺ T cell to beads ratio, followed by the addition of 50 μ L of the effector cells (enriched CD8⁺ cells, 2×10^6 /mL or 1×10^5 /well). The assay plates were incubated for 72 h at 37°C, and culture supernatant harvested. IFN γ levels in the supernatants were measured by ELISA (BD OptiEIA #555142) and are presented in FIG. 36 as normalized data (mean and SD values). EC₅₀ values were determined by

non-linear regression log (agonist) vs. Normalized response (variable slope) using GraphPad Prism 7. TOP value (maximal functional effect) was determined by calculating the mean of the two highest values of IFN γ production in the culture supernatant. The experiment was performed 4 times, including in total 12 donors.

Table 29A: ACN017 Variable Region Sequences

	Heavy chain		Light Chain	
	AA sequence	SEQ ID	AA sequence	SEQ ID
CDR1	GFTFSSYA	30	QSISSY	8
CDR2	ISGSGGST	32	AAS	10
CDR3	AKGSGSYFDL	177	QQYSGYPYT	178
VH/VL	EVQLLES GGGLVQPGGSLRLSC AASGFTFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSV KGRFTISRDN SKNTLYLQMNSL RAEDTAVYYCAKSGSYFDLW GQGTLVTVSS	179	DIQMTQSPSFLSASVGDRV TITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSG VPSRFSGSGSGTDFTLTIS LQPEDFATYYCQQYSGYP YTFGQGTKLEIK	180

Table 29B: Full-length ACN017 Sequence

Full ACN017 Seq (VH-Fc-VL)	SEQ ID
EVQLLES GGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAI SGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKSGS YFDLWGQGT LTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSFLSASV DRV TITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGT DFTLTIS LQPEDFATYYCQQYSGYPYTFGQGTKLEIKSSEPKSSDKTHTCPPC PAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHY TQKSLSLSPGSGGGGSGGGGSGGGGSPSEVQLLES GGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDN SK NTLYLQMNSLRAEDTAVYYCAKSGSYFDLWGQGT LTVSSGGGGSGGGG SGGGGSGGGGSDIQMTQSPSFLSASVGDRV TITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS LQPEDFATYYCQQYSGYP YTFGQGTKLEIKRS	181

Results and conclusions

[00355] The functional effect of the CD137-5T4 bispecific antibody ALG.APV-210 was determined using human CD8+ T cells (the effector cells) co-cultured in plates with HCT116 cells. As shown in FIG. 36, the bispecific CD137-5T4 antibody ALG.APV-210 induced potent T cell activation, measured by IFN γ release, in a dose-dependent manner in the presence of HCT116 cells expressing endogenous levels of 5T4. For determination of EC₅₀, see Table 30, showing mean values and 95% CI of 12 donors from 4 pooled experiments. The endogenous levels of 5T4 expressed by the HCT116 cells is sufficient to induce a high agonistic functional effect of ALG.APV-210 as demonstrated in FIG. 37, where IFN γ levels of 6 individual donors following treatment with ALG.APV-210 is compared with an isotype control (ANC017). The efficacy (maximal IFN γ response) is also presented in FIG. 38 (fold change vs. isotype control, mean and SD of 12 donors).

Table 30: EC₅₀ of ALG.APV-210 in a CD8+ T cell assay in the presence of HCT116 cells expressing human 5T4

Potency of ALG.APV-210	HCT116 T cell assay
EC ₅₀ (nM)	0.17
95% CI	0.14 to 0.21

Example 19: *In vivo* anti-tumor efficacy of a bispecific antibody in HCT-116 a 5T4 positive xenograft tumor model in SCID beige mice

[00356] A study was conducted to assess the anti-tumor efficacy *in vivo* of ALG.APV-210.

Materials and Methods

[00357] Female SCID beige mice (6-8 weeks old) from Taconic, Denmark were used in the experiment. All animal procedures were conducted in compliance with Swedish legislation on animal rights and protection, and approved by the Ethical Committee on Animal Experiments, in Lund/Malmö (ethical application number: M151-13). PBMC were isolated from leukocyte concentrates obtained from 4 healthy donors (Clinical Immunology and Transfusion Medicine, Labmedicin Region Skåne, Lund Sweden) by density gradient centrifugation using

Ficoll-Paque (GE Healthcare #17-1440-02) according to the manufacturer's instructions. The HCT-116 cell line, a human colon carcinoma cell line positive for the tumor-associated antigen 5T4, was purchased from ATCC (ATCC® CCL-247™, lot# 62765668) and cultured according to their recommendations.

[00358] On day 0, HCT-116 tumor cells, growing in log phase, were inoculated subcutaneously into the right hind flank of the mice (3×10^6 in 110 μ L PBS). The following day (day 1), 7×10^6 of human PBMC from 4 donors in 100 μ L of PBS were injected intraperitoneally. Intraperitoneal antibody treatments (100 μ L in PBS) starting on day 6 with either vehicle (PBS), ALG.APV-210 10 μ g/mouse, or ALG.APV-210 100 μ g/mouse were given twice/week for a total of 5 injections (n= 5 mice/ treatment/ donor). Tumor growth was observed and measured twice/week with a caliper in width, length and height of which the tumor volume was calculated ($w/2 \times l/2 \times h/2 \times \pi \times (4/3)$). The experimental endpoint was tumor volume $\geq 2\text{cm}^3$, wounding, or affected health of the mice. Tumor growth was statistically analyzed using Mann-Whitney non-parametric 2-tailed T-test using GraphPad Prism 7 at different time points (day 9, 13, 16, 20 and 23).

Results

[00359] As shown in FIG. 28, ALG.APV-210 at a dose of either 10 μ g/mouse or 100 μ g/mouse showed statistically significant anti-tumor efficacy (*e.g.* tumor volume inhibition) in comparison with the vehicle group from day 13 to 23 (pooled data from 4 donors, for statistical analysis and p-values; see Table 31).

Table 31. Statistical analysis of anti-tumor efficacy following treatment with ALG.APV-210 in an HCT-116 xenograft tumor model

Days following Tumor inoculation	Significant difference ($p < 0.05$) in tumor volume between treatments, Mann-Whitney, non-parametric 2-tailed T test	
	<i>ALG.APV-210 10 μg vs. vehicle</i>	<i>ALG.APV-210 100 μg vs. vehicle</i>
Day 9	No	No
Day 13	Yes * $p = 0.0109$	Yes * $p = 0.0262$
Day 16	Yes*** $p = 0.0008$	Yes** $p = 0.0010$
Day 20	Yes**** $p < 0.0001$	Yes* $p = 0.0009$
Day 23	Yes** $p = 0.0016$	Yes* $p = 0.0225$

[00360] In conclusion, the study showed that ALG.APV-210 had anti-tumoral properties in a 5T4 positive human colon carcinoma xenograft tumor model.

Example 20: Pharmacokinetic Properties of ALG.APV-209 in BALB/c Mice

[00361] A study was conducted to assess the pharmacokinetic (PK) properties of ALG.APV-209 and ALG.APV-210 in mice. Normal BALB/c female mice were injected intravenously (IV) at time 0 with a single dose of 200 µg of ALG.APV-209 or ALG.APV-210 and blood was collected from 1 to 3 mice per time point. Anesthetized mice were exsanguinated via cardiac puncture and serum was collected at t = 15 minutes, and 2, 6, 24, 48, 72, 96, 168, 336 and 504 hours after injection. Concentrations of ALG.APV-209 and ALG.APV-210 in sera were determined with an ELISA method developed to detect only intact protein, using huCD137 ECD-AFH (ALG027) to capture the anti-CD137 BD, and biotinylated 5T4 ECD-mFc (ALG029) to detect the anti-5T4 BD. Estimated PK disposition parameters from non-compartmental analysis (NCA) using Phoenix 64 software (v6.4 WinNonlin™ license) are listed in Table 32, and estimates of half-life along with fit statistics are shown in FIG. 29. A precompiled model for IV dosing with sparse sampling and uniform weighting was used during NCA.

[00362] In normal female BALB/c mice injected IV with a 200 µg bolus dose of ALG.APV-209 or ALG.APV-210, the apparent terminal elimination half-life determined using NCA was 142 and 215 hours, respectively (excluding samples potentially impacted by ADA). Parameter estimates for half-life determined using compartmental analysis (2-compartments with IV dosing) were similar for ALG.APV-209 and ALG.APV-210. NCA estimated clearance and volume for ALG.APV-209 and ALG.APV-210 to be: 0.263 and 0.204 mL/hr/kg, and 54 and 63 mL/kg respectively. By both analysis methods, ALG.APV-210 had the longest terminal elimination half-life and slightly better clearance values. Sudden drops in serum concentrations indicative of anti-drug antibodies (ADA) were seen in late time points for mice dosed with ALG.APV-210, however the presence of ADA could not be confirmed by ELISA methods due to drug still being present in samples, which also likely resulted in rapid clearance of ADA.

Table 32. NCA estimated PK Disposition parameters of ALG.APV-209 and ALG.APV-210

PK Parameter	Units	ALG.APV-209	ALG.APV-210	ALG.APV-210 excluding ADA + sera
Rsq		0.82	0.900	0.966
HL Lambda z	hr	142.0	134.6	215.3
Tmax	hr	0.25	0.25	0.25
Cmax	µg/mL	254.04	252.34	252.34
SE Cmax	µg/mL	N/A	14.6	14.6
Cmax/D	kg*µg/mL/mg	0.025	0.025	0.025
C ₀	µg/mL	262.2	263.6	263.6
AUClast	hr*µg/mL	34858	33054	38705
AUCINF_obs	hr*µg/mL	38043	36317	49124
AUCINF/D_obs	hr*kg*µg/mL/mg	3.804	3.632	4.912
V_obs	mL/kg	53.84	53.46	63.22
CL_obs	mL/hr/kg	0.263	0.275	0.204
Vss/OBS	mL/kg	51.1	51.5	62.7
MRTlast	hr	147.4	136.7	171.9
MRTINF_obs	Hr	194.4	187.2	308.2

- Rsq: Goodness of fit statistic for the terminal elimination phase
- HL Lambda z: Apparent terminal elimination half life
- Tmax: Time of maximum observed concentration
- Cmax: Maximum observed concentration, occurring at Tmax
- SE Cmax: Standard error of the data at Tmax (time of maximum mean concentration)
- Cmax/D: Maximum observed concentration divided by dose
- C₀: Back extrapolated initial concentration at time 0
- _obs: based on the observed concentrations
- AUClast: Area under the curve from the time of dosing until the last measured concentration
- AUCINF: Area under the curve from the time of dosing extrapolated to infinity
- AUCINF/D: AUCINF divided by dose
- V: Volume of distribution based on the terminal phase
- CL: Serum clearance
- Vss: An estimate of the volume of distribution at steady state
- MRTlast: Mean residence time until the last measured concentration
- MRTINF: Mean residence time extrapolated to infinity

Example 21: The Fc portion of ALG.APV-210 does not interact with Fcγ receptors

[00363] ALG.APV-210 contains mutations introduced to prevent interaction with Fcγ receptors (FcγR) that could lead to FcγR-mediated T-cell activation or ADCC, ADCP, CDC etc. Binding of ALG.APV-210 was therefore tested on CHO cell transfectants stably expressing the following FcγR: FcγRI (CD64), FcγRIIa (CD32a His131), FcγRIIb (CD32b), FcγRIIIa (CD16a

Val158 and CD16a Phe158), and FcγRIIIb (CD16b). Untransfected CHO cells were used as negative control. A control human IgG1 molecule was used as a positive control. Different concentrations of ALG.APV-210 or control IgG1 were incubated with the FcγR-expressing cell lines, washed, and labeled with a secondary anti-human IgG reagent. Binding of ALG.APV-210 and the control IgG1 was detected by flow cytometry.

[00364] The results are provided in FIGs. 30A and 30B. FIG. 30B shows that the control IgG1 bound to all the tested FcγR expressing cell lines: FcγRI (CD64), FcγRIIa (CD32a His131), FcγRIIb (CD32b), FcγRIIIa (CD16a Val158 and CD16a Phe158), and FcγRIIIb (CD16b), but not to the untransfected CHO cells (negative control). Significant binding of the control IgG1 to some of the FcγR expressing cell lines were observed at concentrations as low as 4 nM. FIG. 30A shows that no detectable binding was observed with the ALG.APV-210 construct to the tested FcγR: FcγRI (CD64), FcγRIIa (CD32a His131), FcγRIIb (CD32b), FcγRIIIa (CD16a Val158 and CD16a Phe158), and FcγRIIIb (CD16b).

Example 22: *In vitro* activity of a 5T4-CD137 bispecific antibody in a T-cell proliferation assay using human PBMC and CHO-5T4 cells

[00365] The functional activity of the 5T4-CD137 bispecific molecules ALG.APV-209 and ALG.APV-210 was evaluated in a T-cell proliferation assay using PBMC. PBMC were isolated from healthy human blood using standard ficoll gradients. Once isolated, the cells were washed in saline buffer to remove platelets. PBMC were cultured with irradiated CHO-K1/human 5T4 cells, at approximately 120,000 PBMC to 30,000 5T4 (+) cells in 96-well plates. CHO-K1/5T4 cells were irradiated (x-ray Cell Rad irradiator, Faxitron Bioptics, LLC), and washed in medium before plating with PBMC. Anti-CD3 antibody OKT3 (eBioscience) was added to all the wells at a concentration of 1 ng/mL. Cells were cultured in a total volume of 200 μL of RPMI 1640 media (GIBCO) supplemented with 10% fetal bovine serum (SIGMA), sodium pyruvate, antibiotics and non-essential amino acids. Plates were incubated at 37° C, 5% CO₂ in humidified incubators for 72 hours. Supernatants were removed and cells were labeled in the same assay plates with antibodies to CD4, CD8 and CD5 in PBS buffer with 2% BSA and 2 mM EDTA, for 30 min on ice, 7AAD was added to exclude dead cells. After washing cells were resuspended at 120 μL/well and 70 μL/well volumes were collected from each well in a flow cytometer (a LSR-IITM, BD Biosciences). Cell analysis was performed using Flowjo software and analyzed by counting

the number of CD4+ or CD8+ live T cell events (CD4+ CD8- CD5+, 7AAD- or CD4- CD8+ CD5+ 7AAD-) events after gating on lymphocytes using FSC *versus* SSC parameters. Each condition was tested in duplicate and the averages of each duplicate set were graphed using GraphPad Prism 6® graphing and statistics software.

[00366] The results of the study are provided in FIGs. 31A and 31B. Antigen-specific proliferation of CD8+ T cells was evaluated after 72 hrs of stimulation of T cells primed with a low concentration of anti-CD3 antibodies. Anti-CD3 stimulation induces upregulation of CD137. Addition of anti-CD3 alone, with no bispecific antibodies, induced a certain amount of CD8+ and CD4+ T-cell proliferation. The addition of ALG.APV-209 or ALG.APV-210 increased the proliferation of CD8+ T cells (FIG. 31A). In contrast, and as expected based on the preferential expression of CD137 on CD8+ T cells, the bispecific antibodies had a limited impact on the anti-CD3 induced proliferation of CD4+ T cells (FIG. 31B). The results were consistent across multiple experiments. A Morrison format control molecule (ALG.APV-004) was included for comparison.

[00367] Taken together, the results showed that both ALG.APV-209 and ALG.APV-210 enhanced the proliferation of CD8+ T cells. ALG.APV-209 and ALG.APV-210 induced a higher amount of cell proliferation than the Morrison format construct ALG.APV-004.

Example 23: 5T4 expression in normal and tumor tissues assessed by immunohistochemistry

[00368] Expression of the tumor antigen 5T4 in normal human tissue and in a range of different tumor types was assessed by immunohistochemistry (IHC).

[00369] Development of staining method: Five commercial anti-5T4 antibodies and two in-house generated anti-5T4 antibodies were assessed for 5T4-binding in cryosections versus formalin-fixed, paraffin-embedded (FFPE) sections of the control cells and test human tissues. Isotype control staining was also included. A mouse monoclonal antibody (clone: MAB4975, R&D Systems) was selected as the best performing antibody because it was highly specific and functional in both cryosections and in FFPE sections. Antigen retrieval method and antibody titer were optimized for this antibody.

[00370] Tissues and tissue microarrays: Human placental tissue and a murine colon carcinoma cell line CT26 (from ATCC® CRL-2638) transfected to express human 5T4 were used as positive controls. Human cerebrum, liver, and non-transfected CT26 cells were used as negative controls. Human tissues used for positive and negative controls were from Charles River

Laboratories' archives. FFPE tissue microarrays (TMAs) were acquired from US Biomax Inc. All stainings were evaluated by a study pathologist at PAI, Charles River Laboratories.

[00371] IHC staining of normal human tissue microarrays: In the normal human tissue panel with 24 tissue types from 3 donors (FDA808j-1), 5T4 expression was not found in any major organs.

[00372] IHC staining in human tumor tissue types: 5T4 expression was screened in the multiple organ cancer tissue array of 72 tumor cases (FDA808j-2) presented by a single core per tumor. 5T4 expression was detected in tumors of the pancreas, thyroid gland, breast, liver, uterus, cervix, striated muscle, skin (squamous cell carcinoma), nerve (neurofibroma), and bladder.

[00373] 5T4 expression was assessed in TMAs containing 10-50 tumor cases for each of the selected indications shown in Table 33, represented by duplicate or triplicate cores. In bladder cancer, cancer of the head and neck (H&N), non-small cell lung cancer (NSCLC) and mesothelioma, 50-56% of the tumors investigated were 5T4 positive. A significant number of pancreatic tumors were also 5T4 positive (36%). 50% of the clear cell carcinomas tumors investigated were 5T4 positive.

Table 33. 5T4 expression in seven selected tumor indications

	TMA ID	Total No. donors	5T4+donors (Total #)	5T4+donors (%)
Bladder cancer	BL721	18	10	56
Head & neck cancers	HN811	19	10	53
Non-small cell lung cancer	LC10011a	40	20	50
Mesothelioma	MS481c	20	10	50
Pancreatic cancer	PA721a	22	8	36
Kidney cancer	T072b	10	2	20
Ovarian cancer	T112c	10	1	10

INCORPORATION BY REFERENCE

[00374] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However,

mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not, be taken as an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

CLAIMS

1. A multispecific polypeptide comprising a first single chain variable fragment (scFv) domain and a second scFv domain linked together by a binding domain linker and an immunoglobulin Fc domain,

wherein the immunoglobulin Fc domain comprises a hinge region and an immunoglobulin constant region;

wherein the multispecific polypeptide is capable of forming a homodimer by association with a second identical multispecific polypeptide;

wherein the first scFv domain specifically binds to human 5T4 and comprises:

(i) an immunoglobulin heavy chain variable region comprising a heavy chain complementarity determining region (HCDR)-1 amino acid sequence of SEQ ID NO: 30, an HCDR2 amino acid sequence of SEQ ID NO: 32, and an HCDR3 amino acid sequence of SEQ ID NO: 34; and

(ii) an immunoglobulin light chain variable region comprising a light chain complementarity determining region (LCDR)-1 amino acid sequence of SEQ ID NO: 42, an LCDR2 amino acid sequence of SEQ ID NO: 10, and an LCDR3 amino acid sequence of SEQ ID NOs: 36; and

wherein the second scFv domain specifically binds to human 4-1BB and comprises:

(i) an immunoglobulin heavy chain variable region comprising an HCDR1 amino acid sequence of SEQ ID NO: 2, an HCDR2 amino acid sequence of SEQ ID NO: 4, and an HCDR3 amino acid sequence of the SEQ ID NO: 6; and

(ii) an immunoglobulin light chain variable region comprising an LCDR1 amino acid sequence of SEQ ID NO: 8, an LCDR2 amino acid sequence of SEQ ID NO: 10, and an LCDR3 amino acid sequence of SEQ ID NO: 12.

2. The multispecific polypeptide of claim 1, wherein the multispecific polypeptide comprises, from amino-terminus to carboxyl-terminus: (i) the first scFv domain, (ii) the hinge region, (iii) the immunoglobulin constant region, (iv) the binding domain linker, and (v) the second scFv domain.
3. The multispecific polypeptide of claim 1, wherein the first scFv domain comprises a mutation in the framework region compared to the framework region of SEQ ID NOs: 130 or 170.
4. The multispecific polypeptide of claim 3, wherein the mutation introduces a stabilizing disulfide bond.
5. The multispecific polypeptide of claim 1, wherein the first scFv domain comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 38 and the immunoglobulin light chain variable region of SEQ ID NO: 44.
6. The multispecific polypeptide of claim 1, wherein the first scFv domain comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 46 and the immunoglobulin light chain variable region of SEQ ID NO: 48.
7. The multispecific polypeptide of claim 1, wherein the second scFv domain comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 14 and the immunoglobulin light chain variable region of SEQ ID NO: 16.
8. The multispecific polypeptide of claim 1, wherein the first scFv domain comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 46 and the immunoglobulin light chain variable region of SEQ ID NO: 48, and wherein the second scFv domain comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 14 and the immunoglobulin light chain variable region of SEQ ID NO: 16.

9. The multispecific polypeptide of claim 1, wherein the amino acid sequence of the first scFv domain is at least 97% identical to SEQ ID NO: 120, and the amino acid sequence of the second scFv domain is at least 97% identical to SEQ ID NO: 110.
10. The multispecific polypeptide of claim 9, wherein the polypeptide comprises an amino acid sequence at least 95% identical to SEQ ID NO: 172 or comprises an amino acid sequence identical to SEQ ID NO: 172.
11. The multispecific polypeptide of claim 1, wherein the amino acid sequence of the first scFv domain is at least 97% identical to SEQ ID NO: 122 and the amino acid sequence of the second scFv domain is at least 97% identical to SEQ ID NO: 110.
12. The multispecific polypeptide of claim 11, wherein the polypeptide comprises an amino acid sequence at least 95% identical to SEQ ID NO: 174 or comprises an amino acid sequence of SEQ ID NO: 174.
13. The multispecific polypeptide of claim 1, wherein binding of the multispecific polypeptide to an effector cell results in increased effector cell activation, increased effector cell proliferation, or wherein binding of the multispecific polypeptide to an effector cell and a 5T4-expressing cell results in enhanced effector cell-dependent lysis of the 5T4-expressing cell.
14. Use of a therapeutically effective amount of the multispecific polypeptide of claim 1, in the manufacture of a medicament for treating cancer, said cancer is characterised by expression of 5T4.
15. The use as claimed in claim 14, wherein the cancer is breast cancer, pancreatic cancer, ovarian cancer, non-small cell lung cancer, mesothelioma, chronic lymphocytic leukemia (CLL), mantle cell leukemia (MCL), acute lymphoblastic leukemia (ALL), squamous cell carcinoma, melanoma, adrenal cancer, bladder cancer, cervical cancer, renal cancer, gastric cancer, prostate

cancer, thyroid cancer, liver cancer, uterine cancer, neurofibroma, sarcoma, carcinoma, or head and neck cancer.

16. The use as claimed in claim 14, wherein the multispecific polypeptide comprises, from amino-terminus to carboxyl-terminus: (i) the first scFv domain, (ii) the hinge region, (iii) the immunoglobulin constant region, (iv) the binding domain linker, and (v) the second scFv domain.

17. The use as claimed in claim 14, wherein the second scFv domain comprises immunoglobulin heavy chain variable region of SEQ ID NO: 14 and the immunoglobulin light chain variable region of SEQ ID NO: 16, and

wherein the first scFv domain comprises

(i) the immunoglobulin heavy chain variable region of SEQ ID NO: 38 and the immunoglobulin light chain variable region of SEQ ID NO: 44; or

(ii) the immunoglobulin heavy chain variable region of SEQ ID NO: 46 and the immunoglobulin light chain variable region of SEQ ID NO: 48.

18. The use as claimed in claim 14, wherein the amino acid sequence of the second scFv domain of the multispecific polypeptide is at least 97% identical to SEQ ID NO: 110, and wherein the amino acid sequence of the first scFv domain of the multispecific polypeptide is at least 97% identical to SEQ ID NO: 120 or SEQ ID NO: 122.

19. The multispecific polypeptide of claim 1, wherein said polypeptide exhibits statistically significant enhanced effector cell activation compared to a second multispecific polypeptide, wherein the second multispecific polypeptide is an IgG-scFv structure comprising an anti-4-1BB antibody comprising a heavy chain variable region comprising SEQ ID NO: 28 and a light chain variable region comprising SEQ ID NO: 16 and an anti-5T4 scFv comprising a heavy chain

variable region comprising SEQ ID NO: 46 and a light chain variable region comprising SEQ ID NO: 66.

20. The multispecific polypeptide of claim 1, wherein said polypeptide induces statistically significant increased effector cell proliferation compared to a second multispecific polypeptide, wherein the second multispecific polypeptide is an IgG-scFv structure comprising an anti-4-1BB antibody comprising a heavy chain variable region comprising SEQ ID NO: 28 and a light chain variable region comprising SEQ ID NO:16 and an anti-5T4 scFv comprising a heavy chain variable region comprising SEQ ID NO:46 and a light chain variable region comprising SEQ ID NO:66.