761612002340

# APRIL AND BAFF INHIBITORY IMMUNOMODULATORY PROTEINS WITH AND WITHOUT A T CELL INHIBITORY PROTEIN AND METHODS OF USE THEREOF

## Cross Reference to Related Applications

**[0001]** This application claims priority to U.S. provisional application 63/022,373 entitled "APRIL AND BAFF INHIBITORY IMMUNOMODULATORY PROTEINS WITH AND WITHOUT A T CELL INHIBITORY PROTEIN AND METHODS OF USE THEREOF", filed May 8, 2020, to U.S. provisional application 63/034,361, entitled "APRIL AND BAFF INHIBITORY IMMUNOMODULATORY PROTEINS WITH AND WITHOUT A T CELL INHIBITORY PROTEIN AND METHODS OF USE THEREOF", filed June 3, 2020, and to U.S. provisional application 63/080,643, entitled "APRIL AND BAFF INHIBITORY IMMUNOMODULATORY PROTEINS WITH AND WITHOUT A T CELL INHIBITORY PROTEIN AND METHODS OF USE THEREOF", filed September 18, 2020, the contents of each of which are incorporated by reference in their entirety for all purposes.

## Incorporation by Reference of Sequence Listing

**[0002]** The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 761612002340SeqList.TXT, created May 4, 2021, which is 992, 602 bytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

## Field

**[0003]** The present disclosure provides immunomodulatory proteins that exhibit neutralizing activity of BAFF and APRIL (or BAFF/APRIL heterotrimers) alone, or also coupled with inhibition of T cell costimulation. The immunomodulatory proteins include variant domains of B cell maturation antigen (BCMA) alone, and multi-domain immunomodulatory proteins that inhibit B cell responses and also can inhibit T cell costimulation. The present disclosure also provides nucleic acids molecules encoding the immunomodulatory proteins. The immunomodulatory proteins provide therapeutic utility for a variety of immunological diseases or conditions. Compositions and methods for making and using such proteins are provided.

## Background

**[0004]** Modulation of the immune response by intervening in the processes involving interactions between soluble ligands and their receptor is of increasing medical interest. Currently, biologics used to

enhance or suppress immune responses have generally been limited to antibodies (e.g., anti-PD-1 antibodies) or soluble receptors against a single cell surface molecule (e.g., Fc- CTLA-4). Improved therapeutic agents that can modulate the immune response, and particularly B cell immune responses and, in some cases also T cell immune responses, are needed. Provided are embodiments that meet such needs.

## Summary

**[0005]** Provided herein is an immunomodulatory protein that contains at least one T cell inhibitory molecule (TIM) that binds to a T cell stimulatory receptor, or a ligand of a T cell stimulatory receptor; and that antagonizes activity of a T cell stimulatory receptor; and at least one B cell inhibitory molecule (BIM) that binds to a ligand of a B cell stimulatory receptor and/or antagonizes activity of a B cell stimulatory receptor. In some of any embodiments, the immunomodulatory protein contains at least one T cell inhibitory molecule (TIM) that binds to a T cell stimulatory receptor, or a ligand of a T cell stimulatory receptor; or antagonizes activity of a T cell stimulatory receptor; and at least one B cell inhibitory molecule (BIM) that binds to a ligand of a B cell stimulatory receptor and/or antagonizes activity of a B cell stimulatory receptor.

**[0006]** In some of any embodiments, the TIM binds to a ligand of a T cell stimulatory receptor. In some of any embodiments, the T cell stimulatory receptor is CD28; and the ligand of the T cell stimulatory receptor is CD80 or CD86. In some of any embodiments, the T cell stimulatory receptor is CD28; or the ligand of the T cell stimulatory receptor is CD80 or CD86.

**[0007]** In some of any embodiments, the TIM is a CTLA-4 extracellular domain or a binding portion thereof that binds to CD80 or CD86. In some of any embodiments, the CTLA-4 extracellular domain or binding portion thereof consists of the sequence of amino acids set forth in SEQ ID NO:1 or SEQ ID NO:2, a variant CTLA-4 sequence of amino acids that has at least 85% sequence identity to SEQ ID NO:1 or SEQ ID NO:2; or a portion thereof containing an IgV domain. In some of any embodiments, the CTLA-4 extracellular domain consists of the sequence of amino acids set forth in SEQ ID NO:1. In some of any embodiments, the CTLA-4 extracellular domain consists of a variant CTLA-4 sequence of amino acids that has at least 85% sequence identity to SEQ ID NO:1 or a portion thereof containing an IgV domain, wherein the variant sequence comprises one or more amino acid substitutions in SEQ ID NO:1 or the portion thereof containing the IgV domain.

**[0008]** In some of any embodiments, the variant CTLA-4 sequence comprises the amino acid substitution C122S. In some embodiments, the CTLA-4 extracellular domain or the binding portion thereof is set forth in SEQ ID NO: 668.

**[0009]** In some of any embodiments, the variant CTLA-4 binds to the ectodomain of CD80 and CD86, optionally wherein binding affinity to one or both of CD80 and CD86 is increased compared to the sequence set forth in SEQ ID NO:1 or the portion thereof containing the IgV domain.

**[0010]** In some of any embodiments, the one or more amino acid substitutions in a variant CLTA-4 polypeptide comprise amino acid substitutions selected from L12F, R16H, G29W, T53S, M56T, N58S, L63P, L98Q, or Y105L, or combinations thereof. In some embodiments, the one or more amino acid substitutions comprise G29W, L98Q and Y105L. In some embodiments, the one or more amino acid substitutions are G29W/N58S/L63P/Q82R/L98Q/Y105L. In some embodiments, the one or more amino acid substitutions are L12F/R16H/G29W/M56T/L98Q/Y105L. In some embodiments, the one or more amino acid substitutionsare G29W/L98Q/Y105L.

**[0011]** In some of any embodiments, a variant CTLA-4 polypeptide is one in which the variant CTLA-4 polypeptide has at least about 85%, at least about 90% or at least about 95% sequence identity to SEQ ID NO:1, and contains the one or more amino acid substitutions as described.

**[0012]** In some of any embodiments, a variant CTLA-4 polypeptide is one in which the variant CTLA-4 polypeptide has at least about 85%, at least about 90% or at least about 95% sequence identity to SEQ ID NO:2, and contains the one or more amino acid substitutions as described.

**[0013]** In some embodiments, the CTLA-4 extracellular domain or the binding portion thereof is set forth in any one of SEQ ID NO:92, SEQ ID NO:112, SEQ ID NO:165 or SEQ ID NO:186 or a portion thereof comprising the IgV domain.

**[0014]** In some of any embodiments, the variant CTLA-4 consists of the sequence set forth in SEQ ID NO:92 or a portion thereof containing the IgV domain. In some of any embodiments, the variant CTLA-4 consists of the sequence set forth in SEQ ID NO:113 or a portion thereof containing the IgV domain. In some of any embodiments, the variant CTLA-4 consists of the sequence set forth in SEQ ID NO:165 or a portion thereof containing the IgV domain. In some of any embodiments, the variant CTLA-4 consists of the sequence set forth in SEQ ID NO:186 or a portion thereof containing the IgV domain.

**[0015]** In some of any embodiments, the ligand of a B cell stimulatory receptor is APRIL or BAFF; and the B cell stimulatory receptor is TACI, BCMA, or BAFF-receptor. In some of any embodiments, the ligand of a B cell stimulatory receptor is APRIL or BAFF; or the B cell stimulatory receptor is TACI, BCMA, or BAFF-receptor.

**[0016]** In some of any embodiments, the BIM is a TACI polypeptide that consists of the TACI extracellular domain or a binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer. In some embodiments, the BIM is a TACI extracellular domain or the binding portion thereof that has an extracellular domain sequence set forth as (i) the sequence of amino acids set forth in SEQ ID NO:709, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:709; or (iii) a portion of (i) or (ii) comprising one or both of a CRD1 domain and CRD2 domain that

binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer. In some embodiments, the BIM is a TACI extracellular domain or the binding portion thereof that comprises the CRD1 domain and the CRD2 domain.

**[0017]** In some of any embodiments, a BIM that is a TACI polypeptide is one in which the TACI polypeptide is a truncated wild-type TACI extracellular domain that consists of the sequence set forth in SEQ ID NO: 516.

**[0018]** In some of any embodiments, a BIM that is a TACI polypeptide is a truncated wild-type TACI extracellular domain or is a variant thereof, wherein the truncated wild-type TACI extracellular domain contains the cysteine rich domain 2 (CRD2) but lacks the entirety of the cysteine rich domain 1 (CRD1), and/or wherein the variant TACI polypeptide comprises one or more amino acid substitutions in the truncated wild-type TACI extracellular domain.

**[0019]** In some of any embodiments, the a BIM that is a TACI polypeptide is a truncated wild-type TACI extracellular domain or is a variant thereof, wherein the truncated wild-type TACI extracellular domain consists of a contiguous sequence contained within amino acid residues 67-118 that includes amino acid residues 71-104, with reference to positions set forth in SEQ ID NO:709, wherein the variant TACI polypeptide comprises one or more amino acid substitutions in the truncated wild-type TACI extracellular domain.

**[0020]** In some of any embodiments, a BIM that is a TACI polypeptide is one in which the truncated wild-type TACI extracellular domain is 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 59, 50 or 51 amino acids in length. In some of any embodiments, the truncated wild-type TACI extracellular domain consists of amino acid residues 68-110 set forth in SEQ ID NO: 709. In some of any embodiments, the TACI polypeptide consists of the sequence of amino acid set forth in SEQ ID NO:528 or is a variant thereof containing one or more amino acid substitutions in the sequence set forth in SEQ ID NO: 528.

**[0021]** In some of any embodiments, a BIM that is a TACI polypeptide is one in which the TACI polypeptide is a truncated wild-type TACI extracellular domain that consists of the sequence set forth in SEQ ID NO: 528.

**[0022]** In some of any embodiments, a BIM that is a TACI polypeptide is one in which the truncated TACI polypeptide or the variant thereof binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

**[0023]** In some of any embodiments, a BIM that is a TACI polypeptide is one in which the TACI polypeptide is a variant TACI polypeptide, wherein the variant TACI polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the truncated TACI polypeptide.

**[0024]** In some of any embodiments, the variant TACI polypeptide comprises one or more amino acid substitutions in the extracellular domain (ECD) of a reference TACI polypeptide or a specific binding fragment thereof at positions selected from among 74, 75, 76, 77, 78, 79, 82, 83, 84, 85, 86, 87, 88, 92, 95, 97, 98, 99, 101, 102 and 103, corresponding to numbering set forth in SEQ ID NO: 709. In

some of any embodiments, the one or more amino acid substitutions are selected from E74V, Q75E, Q75R, G76S, K77E, F78Y, Y79F, L82H, L82P, L83S, R84G, R84L, R84Q, D85E, D85V, C86Y, I87L, I87M, S88N, I92V, Q95R, P97S, K98T, Q99E, A101D, Y102D, F103S, F103V, F103Y, or a conservative amino acid substitution thereof. In some of any embodiments, the one or more amino acid substitutions comprise at least one of E74V, K77E, Y79F, L82H, L82P, R84G, R84L, R84Q, D85V, or C86Y. In some of any embodiments, the one or more amino acid substitutions are D85E/K98T, I87L/K98T, L82P/I87L, G76S/P97S, K77E/R84L/F103Y, Y79F/Q99E, L83S/F103S, K77E/R84Q, K77E/A101D, K77E/F78Y/Y102D, Q75E/R84Q, Q75R/R84G/I92V, K77E/A101D/Y102D, R84Q/S88N/A101D, R84Q/F103V, K77E/Q95R/A101D or I87M/A101D. In some embodiments, the one or more amino acid substitutions are K77E/F78Y/Y102D. In some embodiments, the one or more amino acid substitutions are Q75E/R84Q. In some embodiments, a BIM that is a TACI polypeptide is the variant TACI polypeptide set forth in SEQ ID NO: 541. In some embodiments, a BIM that is a TACI polypeptide is the variant TACI polypeptide set forth in SEQ ID NO:542.

**[0025]** In some of any embodiments, a BIM that is a TACI polypeptide is one in which the TACI polypeptide is a variant TACI polypeptide that comprises one or more amino acid substitutions in the extracellular domain (ECD) of a reference TACI polypeptide or a specific binding fragment thereof at positions selected from among 40, 59, 60, 61, 74, 75, 76, 77, 78, 79, 82, 83, 84, 85, 86, 87, 88, 92, 95, 97, 98, 99, 101, 102 and 103, corresponding to numbering of positions set forth in SEQ ID NO:709. In some of any embodiments, the one or more amino acid substitutions are selected from W40R, Q59R, R60G, T61P E74V, Q75E, Q75R, G76S, K77E, F78Y, Y79F, L82H, L82P, L83S, R84G, R84L, R84Q, D85E, D85V, C86Y, I87L, I87M, S88N, I92V, Q95R, P97S, K98T, Q99E, A101D, Y102D, F103S, F103V, F103Y, or a conservative amino acid substitution thereof.

**[0026]** In some of any embodiments, the one or more amino acid substitutions comprise at least one of E74V, K77E, Y79F, L82H, L82P, R84G, R84L, R84Q, D85V or C86Y. In some of any embodiments, the one or more amino acid substitution comprise at least the amino acid substitution K77E. In some of any embodiments, the one or more amino acid substitution comprise at least the amino acid substitution R84G. In some of any embodiments, the one or more amino acid substitution comprise at least the amino acid substitution R84Q.

**[0027]** In some of any embodiments, the reference TACI polypeptide is a truncated polypeptide consisting of the extracellular domain of TACI or a specific binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

**[0028]** In some of any embodiments, the reference TACI polypeptide comprises (i) the sequence of amino acids set forth in SEQ ID NO:709, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:709; or (iii) a portion of (i) or (ii) containing one or both of a CRD1 domain and CRD2 domain that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer. In some of any

embodiments, the reference TACI polypeptide lacks an N-terminal methionine. In some of any embodiments, the reference TACI polypeptide comprises the CRD1 domain and the CRD2 domain. In some of any embodiments, the reference TACI polypeptide comprises the sequence set forth in SEQ ID NO:516. In some of any embodiments, the reference TACI polypeptide consists of the sequence set forth in SEQ ID NO:516. In some of any embodiments, the reference TACI polypeptide consists essentially of the CRD2 domain. In some of any embodiments, the reference TACI polypeptide comprises the sequence set forth in SEQ ID NO:528. In some of any embodiments, the reference TACI polypeptide consists of the sequence set forth in SEQ ID NO:528.

**[0029]** In some of any embodiments, the one or more amino acid substitutions are D85E/K98T, I87L/K98T, R60G/Q75E/L82P, R60G/C86Y, W40R/L82P/F103Y, W40R/Q59R/T61P/K98T, L82P/I87L, G76S/P97S, K77E/R84L/F103Y, Y79F/Q99E, L83S/F103S, K77E/R84Q, K77E/A101D, K77E/F78Y/Y102D, Q75E/R84Q, Q75R/R84G/I92V, K77E/A101D/Y102D, R84Q/S88N/A101D, R84Q/F103V, K77E/Q95R/A101D or I87M/A101D. In some of any embodiments, the one or more amino acid substitutions are K77E/F78Y/Y102D. In some of any embodiments, the one or more amino acid substitutions are Q75E/R84Q.

**[0030]** In some of any embodiments, a BIM that that is a TACI polypeptide is one in which the variant TACI polypeptide has at least about 85%, at least about 90% or at least about 95% sequence identity to SEQ ID NO:709, and contains the one or more amino acid substitutions as descrbed.

**[0031]** In some of any embodiments, a BIM that that is a TACI polypeptide is one in which the variant TACI polypeptide has at least about 85%, at least about 90% or at least about 95% sequence identity to SEQ ID NO:719, and contains the one or more amino acid substitutions as descrbed.

**[0032]** In some of any embodiments, a BIM that that is a TACI polypeptide is one in which the variant TACI polypeptide has at least about 85%, at least about 90% or at least about 95% sequence identity to SEQ ID NO:718, and contains the one or more amino acid substitutions as descrbed.

**[0033]** In some of any embodiments, a BIM that that is a TACI polypeptide is one in which the variant TACI polypeptide has at least about 85%, at least about 90% or at least about 95% sequence identity to SEQ ID NO:516, and contains the one or more amino acid substitutions as descrbed.

**[0034]** In some of any embodiments, a BIM that that is a TACI polypeptide is one in which the variant TACI polypeptide has at least about 85%, at least about 90% or at least about 95% sequence identity to SEQ ID NO:528, and contains the one or more amino acid substitutions as descrbed.

**[0035]** In some of any embodiments, a BIM that is a TACI polypeptide is one in which the variant TACI polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the reference TACI polypeptide. In some of any embodiments, the variant TACI polypeptide has increased binding affinity to APRIL. In some of any embodiments, the variant TACI polypeptide has increased binding affinity to BAFF. In some of any embodiments, the variant TACI polypeptide has increased

binding affinity to APRIL and BAFF. In some of any embodiments, the increased binding affinity for BAFF or APRIL is independently increased more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold or 60-fold.

**[0036]** In some of any embodiments, a BIM that is a TACI polypeptide is one in which the variant TACI polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 517-527, 536, 537, 682-701. In some of any embodiments, a BIM that is a TACI polypeptide is one in which the variant TACI polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 529-535, 538-550, or 673-681. In some of any embodiments, a BIM that is a TACI polypeptide is one in which the variant TACI polypeptide consists or consists essentially of the sequence set forth in any one of SEQ ID NOS: 517-527, 536, 537, 682-701. In some of any embodiments, a BIM that is a TACI polypeptide is one in which the variant TACI polypeptide consists or consists essentially of the sequence set forth in any one of SEQ ID NOS: 529-535, 538-550, 673-681.

**[0037]** In some of any embodiments, a BIM is a TACI polypeptide in which the variant TACI polypeptide is is set forth in any one of SEQ ID NO:535, SEQ ID NO: 541, SEQ ID NO:542, or SEQ ID NO:688.

**[0038]** In some of any embodiments, a BIM is a TACI polypeptide is one in which the variant TACI polyeptide consists or consists essentially of the sequence set forth in SEQ ID NO:535. In some of any embodiments, a BIM that is a TACI polypeptide is one in which the variant TACI polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 541. In some of any embodiments, a BIM that is a TACI polypeptide is one in which the variant TACI polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:542. In some of any embodiments, a BIM that is a TACI polypeptide is one in which the variant TACI polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:688. In some of any embodiments, a BIM that is a TACI polypeptide is one in which the variant TACI polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:535.

**[0039]** In some of any embodiments, the BIM is a BCMA polypeptide that consists of the BCMA extracellular domain or a binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer. In some of any embodiments, the BCMA extracellular domain or the binding portion thereof is an extracellular domain sequence set forth as (i) the sequence of amino acids set forth in SEQ ID NO:356, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:356; or (iii) a portion of (i) or (ii) comprising a CRD domain.

**[0040]** In some of any embodiments, the BCMA polypeptide consists of the sequence set forth in SEQ ID NO:356. In some of any embodiments, the BCMA polypeptide is a variant BCMA polypeptide containing one or more amino acid substitutions in the extracellular domain (ECD) of a reference BCMA polypeptide or a specific binding fragment at positions selected from among 9, 10, 11, 14, 16, 19, 20, 22,

25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 and 48, corresponding to numbering set forth in SEQ ID NO: 710.

**[0041]** Provided herein is an immunomodulatory protein containing a variant BCMA polypeptide, wherein the variant BCMA polypeptide comprises one or more amino acid substitutions in the extracellular domain (ECD) of a reference BCMA polypeptide or a specific binding fragment thereof at positions selected from among 9, 10, 11, 14, 16, 19, 20, 22, 25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 and 48, corresponding to numbering of positions set forth in SEQ ID NO:710. In some of any embodiments, the reference BCMA polypeptide is a polypeptide consisting of the extracellular domain of BCMA or a specific binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer. In some of any embodiments, the reference BCMA polypeptide comprises (i) the sequence of amino acids set forth in SEQ ID NO:710, (ii) a sequence of amino acids that has at least 95% 37a.equence identity to SEQ ID NO:710; or (iii) a portion of (i) or (ii) containing the CRD. In some of any embodiments, the reference BCMA lacks an N-terminal methionine.

**[0042]** In some of any embodiments, the reference BCMA polypeptide comprises (i) the sequence of amino acids set forth in SEQ ID NO:356, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:356; or (iii) a portion of (i) or (ii) containing the CRD. In some of any embodiments, the reference BCMA polypeptide comprises the sequence set forth in SEQ ID NO:356. In some of any embodiments, the reference BCMA polypeptide consists of the sequence set forth in SEQ ID NO:356.

**[0043]** In some of any embodiments, the one or more amino acid substitutions of a BCMA polypeptide are selected from S9G, S9N, S9Y, Q10E, Q10P, N11D, N11S, F14Y, S16A, H19A, H19C, H19D, H19E, H19F, H19G, H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R, H19S, H19T, H19V, H19W, H19Y, A20T, I22L, I22V, Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y, R27H, R27L, S29P, S30G, S30Y, N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y, T32I, T32S, L35A, L35M, L35P, L35S, L35V, L35Y, T36A, T36G, T36N, T36M, T36S, T36V, R39L, R39Q, A43E, A43S, V45A, V45D, V45I, T46A, T46I, N47D, N47Y, S48G, or a conservative amino acid substitution thereof.

**[0044]** In some of any embodiments, the one or more amino acid substitutions of a BCMA polypeptide comprise at least one substitution at position 19. In some embodiments, at least one substitution is selected from H19A, H19C, H19D, H19E, H19F, H19G, H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R, H19S, H19T, H19V, H19W, H19Y. In some of any embodiments, the one or more amino acid substitution comprise at least the amino acid substitution H19L. In some of any embodiments, the one or more amino acid substitution comprise at least the amino acid substitution H19K. In some of any embodiments, the one or more amino acid substitution comprise at least the amino

acid substitution H19R. In some of any embodiments, the one or more amino acid substitution comprise at least the amino acid substitution H19Y.

**[0045]** In some of any embodiments, the one or more amino acid substitutions of a BCMA polypeptide comprise at least one substitution at position 25. In some embodiments, at least one substitution is selected from Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y.

**[0046]** In some of any embodiments, the one or more amino acid substitutions of a BCMA polypeptide comprise at least one substitution at position 31. In some embodiments, at least one substitution is selected from N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y.

**[0047]** In some of any embodiments, the one or more amino acid substitutions of a BCMA polypeptide comprise at least one substitution at position 35. In some embodiments, at least one substitution is selected from L35A, L35M, L35P, L35S, L35V, L35Y.

**[0048]** In some of any embodiments, the one or more amino acid substitutions of a BCMA polypeptide comprise at least one substitution at position 36. In some embodiments, at least one substitution is selected from T36A, T36G, T36N, T36M, T36S, T36V.

**[0049]** In some of any embodiments, the one or more amino acid substitutions of a BCMA polypeptide are H19Y/S30G; H19Y/V45A; F14Y/H19Y; H19Y/V45D; H19Y/A43E; H19Y/T36A; H19Y/I22V; N11D/H19Y; H19Y/T36M; N11S/H19Y; H19Y/L35P/T46A; H19Y/N47D; S9D/H19Y; H19Y/S30G/V45D; H19Y/R39Q; H19Y/L35P; S9D/H19Y/R27H; Q10P/H19Y/Q25H; H19Y/R39L/N47D; N11D/H19Y/N47D; H19Y/T32S; N11S/H19Y/S29P; H19Y/R39Q/N47D; S16A/H19Y/R39Q; S9N/H19Y/N31K/T46I; H19Y/R27L/N31Y/T32S/T36A; N11S/H19Y/T46A; H19Y/T32I; S9G/H19Y/T36S/A43S; H19Y/S48G; S9N/H19Y/I22V/N31D; S9N/H19Y/Q25K/N31D; S9G/H19Y/T32S; H19Y/T36A/N47Y; H19Y/V45A/T46I; H19Y/Q25K/N31D; H19Y/Q25H/R39Q/V45D; H19Y/T32S/N47D; Q10E/H19Y/A20T/T36S; H19Y/T32S/V45I; H19F/Q25E/N31L/L35Y/T36S; H19F/Q25F/N31S/T36S; H19I/Q25F/N31S/T36V; H19F/Q25V/N31M/T36S; H19Y/Q25Y/N31L/L35Y/T36S; H19F/Q25I/N31M/L35A/T36S; H19I/Q25L/N31L/L35Y/T36S; H19F/Q25L/N31G/L35P/T36A; H19Y/I22L/N31G; H19F/I22V/Q25M/N31P/T36M; H19Y/N31L/L35Y/T36S; H19L/S30G/N31H/L35A; H19L/Q25S/N31V/L35S/T36V; H19L/Q25S/S30Y/N31G/L35M/T36V; H19F/Q25F/N31L/L35Y/T36S; H19F/Q25F/N31S/T36G; H19F/I22V/Q25S/N31V/L35S/T36V; H19F/Q25G/N31S/L35V/T36N; H19L/Q25H/N31D/L35S; or H19F/Q25F/N31S/L35Y/T36S.

**[0050]** In some of any embodiments, the one or more amino acid substitutions of a BCMA polypeptide comprise H19F, H19L, H19K, H19M, H19R, H10Y, N11D/H19Y/N47D, H19Y/R39Q/N47D; S16A/H19Y/R39Q, S9G/H19Y/T32S; H19Y/T36A/N47Y; or

Q10E/H19Y/A20T/T36S. In some of any embodiments, the one or more amino acid substitutions of a BCMA polypeptide comprise S16A/H19Y/R39Q.

**[0051]** In some of any embodiments, a variant BCMA polypeptide is one in which the variant BCMA polypeptide has at least about 85%, at least about 90% or at least about 95% sequence identity to SEQ ID NO:710, and contains the one or more amino acid substitutions as described.

**[0052]** In some of any embodiments, a variant BCMA polypeptide is one in which the variant BCMA polypeptide has at least about 85%, at least about 90% or at least about 95% sequence identity to SEQ ID NO:356, and contains the one or more amino acid substitutions as described.

**[0053]** In some of any embodiments, the variant BCMA polypeptide has up to 10 amino acid substitutions compared to the reference BCMA polypeptide. In some of any embodiments, the variant BCMA polypeptide has up to 5 amino acid substitutions compared to the reference BCMA polypeptide. In some of any embodiments, the variant BCMA polypeptide has at least 90% sequence identity to SEQ ID NO:356. In some of any embodiments, the variant BCMA polypeptide has at least 95% sequence identity to SEQ ID NO:356.

**[0054]** In some of any embodiments, the variant BCMA polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the reference BCMA polypeptide. In some of any embodiments, the variant BCMA polypeptide has increased binding affinity to APRIL. In some of any embodiments, the variant BCMA polypeptide has increased binding affinity to BAFF. In some of any embodiments, the variant BCMA polypeptide has increased binding affinity to APRIL and BAFF. In some of any embodiments, the increased binding affinity for BAFF or APRIL is independently increased more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold or 60-fold.

**[0055]** In some of any embodiments, the variant BCMA polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 357-435. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in any one of SEQ ID NOS: 357-435. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 357. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 377. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 380. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 381. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 390. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 391. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 396. In some of any embodiments, the variant

BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 402. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 405. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 406. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 407. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:411. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:405. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:406.

**[0056]** In some of any embodiments, the immunomodulatory protein contains a heterologous moiety that is linked to the at least one BCMA polypeptide. In some of any embodiments, the heterologous moiety is a half-life extending moiety, a multimerization domain, a targeting moiety that binds to a molecule on the surface of a cell, or a detectable label. In some of any embodiments, the half-life extending moiety comprises a multimerization domain, albumin, an albumin-binding polypeptide, Pro/Ala/Ser (PAS), a C-terminal peptide (CTP) of the beta subunit of human chorionic gonadotropin, polyethylene glycol (PEG), long unstructured hydrophilic sequences of amino acids (XTEN), hydroxyethyl starch (HES), an albumin-binding small molecule, or a combination thereof.

**[0057]** In some of any embodiments, the immunomodulatory protein contains an Fc region of an immunoglobulin that is linked to the at least one BCMA polypeptide.

**[0058]** In some of any embodiments, the at least one TIM comprises only one TIM. In some of any embodiments, the at least one TIM comprises 2, 3, 4, or 5 TIMs, optionally wherein each TIM is the same. In some of any embodiments, each TIM is linked directly or indirectly via a linker, optionally wherein the linker is a peptide linker. In some of any embodiments, the at least one BIM comprises only one BIM. In some of any embodiments, the at least one BIM comprises 2, 3, 4, or 5 BIMs, optionally wherein each BIM is the same. In some of any embodiments, each BIM is linked directly or indirectly via a linker, optionally wherein the linker is a peptide linker.

**[0059]** In some of any embodiments, the linker is a peptide linker and the peptide linker is selected from GSGGS (SEQ ID NO: 592), GGGGS (G4S; SEQ ID NO: 593), GSGGGGS (SEQ ID NO: 590), GGGGSGGGGS (2xGGGGS; SEQ ID NO: 594), GGGGSGGGGSGGGGS (3xGGGGS; SEQ ID NO: 595), GGGGSGGGGSGGGGSGGGGS (4xGGGGS, SEQ ID NO:600), GGGGSGGGGSGGGGSGGGGSGGGGS (5XGGGGS, SEQ ID NO: 671), GGGGSSA (SEQ ID NO: 596) or combinations thereof.

**[0060]** In some of any embodiments, the at least one TIM and the at least one BIM are linked directly or indirectly via a linker, optionally wherein the linker comprises a peptide linker and/or a

multimerization moiety. In some of any embodiments, the linker comprises a peptide linker and the peptide linker is selected from GSGGS (SEQ ID NO: 592), GGGGS (G4S; SEQ ID NO: 593), GSGGGGS (SEQ ID NO: 590), GGGGSGGGGS (2xGGGGS; SEQ ID NO: 594), GGGGSGGGGSGGGGS (3xGGGGS; SEQ ID NO: 595), GGGGSGGGGSGGGGSGGGGS (4xGGGGS, SEQ ID NO:600), GGGGSGGGGSGGGGSGGGGSGGGGS (5XGGGGS, SEQ ID NO: 671), GGGGSSA (SEQ ID NO: 596) or combinations thereof. In some of any embodiments, the linker comprises a peptide linker and the peptide linker is selected from SEQ ID NO: 711 (1xEAAAK), SEQ ID NO: 712 (2xEAAAK), SEQ ID NO: 713 (3xEAAAK), SEQ ID NO: 714 (4xEAAAK), SEQ ID NO: 715 (5xEAAAK), SEQ ID NO: 665 (6xEAAAK). In some of any embodiments, the immunomodulatory protein is a monomer and/or comprises a single polypeptide chain.

**[0061]** In some of any embodiments, the at least one TIM is amino-terminal to the at least one BIM in the polypeptide. In some of any embodiments, the at least one TIM is carboxy-terminal to the at least one BIM in the polypeptide.

**[0062]** In some of any embodiments, the immunomodulatory protein further comprises a detectable label, optionally wherein the detectable label is a Flag tag, a His tag, or a myc tag. In some of any embodiments, the immunomodulatory protein comprises the sequence of amino acids set forth in any of SEQ ID NOS: 618-623, or a sequence that exhibits at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto and retains activity.

**[0063]** In some of any embodiments, the immunomodulatory protein comprises the sequence of amino acids set forth in any of SEQ ID NOS: 703-708, or a sequence that exhibits at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto and retains activity.

**[0064]** In some embodiments, the TIM and the BIM are linked by a multimerization domain. In some of any embodiments, the multimerization domain promotes dimerization, trimerization, tetramerization, or pentamerization. In some of any embodiments, the multimerization domain is an immunoglobulin Fc region. In some of any embodiments, the immunomodulatory protein is a dimer. In some of any embodiments, the immunoglobulin Fc region is a homodimeric Fc region. In some of any embodiments, the immunoglobulin Fc region is a heterodimeric Fc region.

**[0065]** In some of any embodiments, the immunomodulatory protein is a homodimer, wherein each polypeptide of the dimer is the same. In some of any embodiments, each polypeptide comprises the at least one TIM and the at least one BIM and wherein the at least one TIM is amino-terminal to the at least one BIM in each polypeptide. In some of any embodiments, each polypeptide comprises the at least one TIM and the at least one BIM and wherein the at least one TIM is carboxy-terminal to the at least one BIM in each polypeptide.

**[0066]** In some of any embodiments, the immunoglobulin Fc region is an IgG2 Fc domain. In some embodiment, the IgG2 Fc domain comprises the sequence of amino acids set forth in SEQ ID NO: 729 or 853 or a sequence of amino acids that exhibits at least 95% sequence identity to SEQ ID NO:729 or 853. In some embodiments, the IgG2 Fc domain is set forth in SEQ ID NO:729. In some embodiments, the IgG2 Fc domain is set forth in SEQ ID NO: 853.

**[0067]** In some of any embodiments, the immunoglobulin Fc region is an IgG4 Fc domain. In some of any embodiments the IgG4 Fc domain is a variant IgG4 Fc domain comprising the amino acid substitution S228P. In some embodiemnts, the IgG4 Fc domain comprises the sequence of amino acids set forth in SEQ ID NO: 731 or 854, or a sequence of amino acids that exhibits at least 95% sequence identity to SEQ ID NO: 731 or 854. In some embodiments, the IgG4 Fc domain is set forth in SEQ ID NO:731. In some embodiments, the IgG4 Fc domain is set forth in SEQ ID NO: 854.

**[0068]** In some of any embodiments, the immunoglobulin Fc is an IgG1 Fc domain, or is a variant Fc that exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, optionally as compared to a wild-type IgG1 Fc domain. In some embodiments, the immunoglobulin Fc is an IgG1 Fc domain and the Fc includes the amino acid sequence set forth in SEQ ID NO: 597. In some of any embodiments, the immunoglobulin Fc is an IgG4 Fc domain, either wild type or modified.

**[0069]** In some of any embodiments, the immunoglobulin Fc is a variant IgG1 Fc domain containing one or more amino acid substitutions selected from L234A, L234V, L235A, L235E, G237A, S267K, R292C, N297G, and V302C, by EU numbering. In some of any embodiments, the immunoglobulin Fc region contains the amino acid substitutions L234A, L235E an G237A by EU numbering or the amino acid substitutions R292C, N297G and V302C by EU numbering. In some embodiments, the Fc is a variant Fc including the amino acid sequence set forth in SEQ ID NO:589. In some embodiments, the Fc is a variant Fc including the amino acid sequence set forth in SEQ ID NO: 855.

**[0070]** In some embodiments, the immunomodulatory protein is a BCMA-Fc fusion proteion. In some of any embodiments, the BCMA-Fc fusion protein comprises the structure: BCMA polypeptide (BCMA)-Linker-Fc region. In some embodiments, the BCMA-Fc fusion protein is set forth in SEQ ID NO:629.

**[0071]** Also provided herein is an immunomodulatory BCMA-Fc fusion protein that is a homodimer comprising two identical copies of the BCMA-Fc fusion protein set forth in SEQ ID NO: 629 linked by a covalent disulfide bond.

**[0072]** In some of any embodiments, the immunomodulatory protein is a BCMA-Fc fusion protein that has the structure: (BCMA)-Linker-Fc region-Linker-(BCMA). In some embodiments, the BCMA-Fc fusion protein is set forth in SEQ ID NO: 809. In some embodiments, the BCMA-Fc fusion protein is set forth in SEQ ID NO: 812.

**[0073]** In some of any embodiments, the BCMA-Fc fusion protein has the structure: (BCMA)-Linker-(BCMA)-Linker-Fc region. In some embodiments, the BCMA-Fc fusion protein is set forth in SEQ ID NO: 813.

**[0074]** In some of any embodiments, the immunomodulatory protein that contains at least one TIM and at least one BIM comprises the sequence of amino acids set forth in any of SEQ ID NOS: 610-617, 624-627, 637, 638, 643, 644, 648, 653 and 654, or a sequence that exhibits at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto and retains activity.

**[0075]** In some embodiments, the TIM is a wild-type CTLA-4 extracellular domain or a binding portion thereof, and the BIM is a TACI extracellular domain or a binding portion thereof comprising amino acid substitutions K77E/F78Y/Y102D, Q75E/R84Q, or R84G, corresponding to positions set forth in SEQ ID NO:709. In some embodiments, the TIM is set forth in SEQ ID NO:1 and the BIM is set forth in SEQ ID NO:535, 541, 542, or 688. In some embodiemnts, the immunomodulatory protein comprises the sequence set forth in SEQ ID NO:611, SEQ ID NO:788, SEQ ID NO: 789, SEQ ID NO: 790, or SEQ ID NO: 792.

**[0076]** Provided herein is an immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 611 linked by a covalent disulfide bond.

**[0077]** Provided herein is an immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 788 linked by a covalent disulfide bond.

**[0078]** Provided herein is an immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 789 linked by a covalent disulfide bond.

**[0079]** Provided herein is an immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 790 linked by a covalent disulfide bond.

**[0080]** Provided herein is an immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 792 linked by a covalent disulfide bond.

**[0081]** In some of any embodiments, the TIM is a wild-type CTLA-4 extracellular domain or a binding portion thereof, and the BIM is a truncated TACI extracellular domain comprising the CRD2 domain. In some embodiments, the TIM is set forth in SEQ ID NO:1 and the BIM is set forth in SEQ ID NO:528. In some embodiments, the immunomodulatory protein comprises the sequence set forth in SEQ ID NO:759, SEQ ID NO: 853, SEQ ID NO: 854 or SEQ ID NO: 791.

**[0082]** Provided herein is an immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 759 linked by a covalent disulfide bond.

**[0083]** Provided herein is an immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 853 linked by a covalent disulfide bond.

**[0084]** Provided herein is an immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 854 linked by a covalent disulfide bond.

**[0085]** Provided herein is an immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 791 linked by a covalent disulfide bond.

**[0086]** In some of any embodiments, the TIM is a CTLA-4 extracellular domain or a binding portion thereof, comprising amino acid substitutions G29W/L98Q/Y105L corresponding to positions set forth in SEQ ID NO:1, and the BIM is a TACI extracellular domain or a binding portion thereof comprising amino acid substitutions K77E/F78Y/Y102D, Q75E/R84Q, or R84G, corresponding to positions set forth in SEQ ID NO:709. In some embodiments, the TIM is set forth in SEQ ID NO:186 and the BIM is set forth in SEQ ID NO:535, 541, 542, or 688. In some embodiments, the immunomodulatory protein comprises he sequence set forth in SEQ ID NO:610.

**[0087]** Provided herein is an immunomodulatory protein comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 610 linked by a covalent disulfide bond.

**[0088]** In some of any embodiments, the immunomodulatory protein that contains at least one TIM and at least one BIM comprises the sequence of amino acids set forth in any of SEQ ID NOS: 601-609, 631-636, 645-647, 649-652, 655-659, or a sequence that exhibits at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto and retains activity.

**[0089]** In some of any embodiments, the TIM is a wild-type CTLA-4 extracellular domain or a binding portion thereof, and the BIM is a BCMA extracellular domain or a binding portion thereof comprising the amino acid substitution H19L corresponding to positions set forth in SEQ ID NO:710. In some embodiments, the TIM is set forth in SEQ ID NO:1 and the BIM is set forth in SEQ ID NO:406. In some embodiments, the immunomodulatory protein comprises the sequence set forth in SEQ ID NO:602.

**[0090]** Provided herein is an immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 602 linked by a covalent disulfide bond.

**[0091]** In some of any embodiments, the TIM is a CTLA-4 extracellular domain or a binding portion thereof comprising the amino acid substitutions G29W/L98Q/Y105L corresponding to positions set forth in SEQ ID NO:1, and the BIM is a BCMA extracellular domain or a binding portion thereof comprising the amino acid substitution H19L with reference to positions set forth in SEQ ID NO:710. In some embodiments, the TIM is set forth in SEQ ID NO:186 and the BIM is set forth in SEQ ID NO:406. In some embodiments, the immunomodulatory protein comprises the sequence set forth in SEQ ID NO:601.

**[0092]** Provided herein is an immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 601 linked by a covalent disulfide bond.

**[0093]** In some of any embodiments, the immunomodulatory protein is a heterodimer, wherein each polypeptide of the dimer is linked to an immunoglobulin Fc domain individually containing one or more amino acid modifications in a wild-type Fc domain to effect heterodimer formation between the polypeptides. In some of any embodiments, the wild-type immunoglobulin Fc is an IgG1 Fc domain. In some of any embodiments, the one more amino acid modifications are selected from a knob-into-hole modification and a charge mutation to reduce or prevent self-association due to charge repulsion. In some of any embodiments, the Fc region further contains one or more amino acid substitutions to reduce binding affinity to an Fc receptor and/or reduce effector function, optionally as compared to a wild-type IgG1 Fc domain. In some of any embodiments, the one or more amino acid substitutions are selected from L234A, L234V, L235A, L235E, G237A, S267K, R292C, N297G, and V302C, by EU numbering. In some of any embodiments, the immunoglobulin Fc region contains the amino acid substitutions L234A, L235E an G237A by EU numbering or the amino acid substitutions R292C, N297G and V302C by EU numbering.

**[0094]** In some of any embodiments, the heterodimer comprises a first polypeptide containing the sequence of amino acids set forth in SEQ ID NO: 662 or 663 and a second polypeptide containing the sequence of amino acids set forth in SEQ ID NO:660.

**[0095]** In some of any embodiments, the immunomodulatory protein blocks binding of APRIL, BAFF, or an APRIL/BAFF heterotrimer to BCMA or TACI; and the immunomodulatory protein reduces the levels of circulating APRIL, BAFF, or an APRIL/BAFF in the blood following administration to a subject. In some of any embodiments, the immunomodulatory protein blocks binding of APRIL, BAFF, or an APRIL/BAFF heterotrimer to BCMA or TACI; or the immunomodulatory protein reduces the levels of circulating APRIL, BAFF, or an APRIL/BAFF in the blood following administration to a subject. In some of any embodiments, the immunomodulatory protein reduces or inhibits B cell maturation, differentiation and proliferation.

**[0096]** In some of any embodiments, the immunomodulatory protein blocks binding of CD80 or CD86 to a costimulatory receptor, optionally wherein the costimulatory receptor is CD28; and the immunomodulatory protein reduces or inhibits T cell co-stimulation. In some of any embodiments, the immunomodulatory protein reduces or inhibits B cell maturation, differentiation or proliferation. In some of any embodiments, the immunomodulatory protein blocks binding of CD80 or CD86 to a costimulatory receptor, optionally wherein the costimulatory receptor is CD28; or the immunomodulatory protein reduces or inhibits T cell costimulation.

**[0097]** Provided herein is a nucleic acid molecule(s) encoding the immunomodulatory protein of any of the embodiments described herein. In some of any embodiments, the nucleic acid molecule is a synthetic nucleic acid. In some of any embodiments, the nucleic acid molecule is a cDNA.

**[0098]** Provided herein is a vector, containing the nucleic acid molecule of any of the embodiments described herein. In some of any embodiments, the vector is an expression vector. In some of any embodiments, the vector is a mammalian expression vector or a viral vector.

**[0099]** Provided herein is a cell, containing the nucleic acid of any of any of the embodiments described herein or the vector of any of any of embodiments described herein. In some of any embodiments, the cell is a mammalian cell. In some of any embodiments, the cell is a human cell.

**[0100]** Provided herein is a method of producing an immunomodulatory protein, including introducing the nucleic acid molecule of any of the embodiments described herein or vector of any of the embodiments described herein into a host cell under conditions to express the protein in the cell. In some of any embodiments, the method includes isolating or purifying the immunomodulatory protein from the cell.

**[0101]** Provided herein is an immunomodulatory protein produced by the method of any of the embodiments described herein.

**[0102]** Provided herein is a pharmaceutical composition, including the immunomodulatory protein of any of the embodiments described herein.

**[0103]** Provided herein is a variant BCMA-Fc fusion protein including a variant BCMA polypeptide, an Fc region, and a linker between the BCMA polypeptide and Fc region, wherein the variant BCMA polypeptide comprises one or more amino acid substitutions in the extracellular domain (ECD) of an unmodified BCMA polypeptide or a specific binding fragment thereof corresponding to positions selected from among 9, 10, 11, 14, 16, 19, 20, 22, 25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 and 48 with reference to positions set forth in SEQ ID NO:710.

**[0104]** In some of any embodiments, the reference BCMA polypeptide is a polypeptide consisting of the extracellular domain of BCMA or a specific binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer. In some of any embodiments, the reference BCMA polypeptide comprises (i) the sequence of amino acids set forth in SEQ ID NO:710, (ii) a sequence of amino acids that has at least 95% 37a.equence identity to SEQ ID NO:710; or (iii) a portion of (i) or (ii) containing the CRD. In some of any embodiments, the reference BCMA lacks an N-terminal methionine.

**[0105]** In some of any embodiments, the reference BCMA polypeptide comprises (i) the sequence of amino acids set forth in SEQ ID NO:356, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:356; or (iii) a portion of (i) or (ii) containing the CRD. In some of any embodiments, the reference BCMA polypeptide comprises the sequence set forth in SEQ ID NO:356. In some of any embodiments, the reference BCMA polypeptide consists of the sequence set forth in SEQ ID NO:356.

**[0106]** In some of any embodiments, the one or more amino acid substitutions are selected from S9G, S9N, S9Y, Q10E, Q10P, N11D, N11S, F14Y, S16A, H19A, H19C, H19D, H19E, H19F, H19G,

H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R, H19S, H19T, H19V, H19W, H19Y, A20T, I22L, I22V, Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y, R27H, R27L, S29P, S30G, S30Y, N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y, T32I, T32S, L35A, L35M, L35P, L35S, L35V, L35Y, T36A, T36G, T36N, T36M, T36S, T36V, R39L, R39Q, A43E, A43S, V45A, V45D, V45I, T46A, T46I, N47D, N47Y, S48G, or a conservative amino acid substitution thereof. In some of any embodiments, the one or more amino acid substitutions comprise at least one substitution at position 19, optionally wherein the at least one substitution is selected from H19A, H19C, H19D, H19E, H19F, H19G, H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R, H19S, H19T, H19V, H19W, H19Y. In some of any embodiments, the one or more amino acid substitution comprise at least the amino acid substitution H19L. In some of any embodiments, the one or more amino acid substitution comprise at least the amino acid substitution H19K. In some of any embodiments, the one or more amino acid substitution comprise at least the amino acid substitution H19R. In some of any embodiments, the one or more amino acid substitution comprise at least the amino acid substitution H19Y.

**[0107]** In some of any embodiments, the one or more amino acid substitutions comprise at least one substitution at position 25, optionally wherein the at least one substitution is selected from Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y. In some of any embodiments, the one or more amino acid substitutions comprise at least one substitution at position 31, optionally wherein the at least one substitution is selected from N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y.

**[0108]** In some of any embodiments, the one or more amino acid substitutions comprise at least one substitution at position 35, optionally wherein the at least one substitution is selected from L35A, L35M, L35P, L35S, L35V, L35Y. In some of any embodiments, the one or more amino acid substitutions comprise at least one substitution at position 36, optionally wherein the at least one substitution is selected from T36A, T36G, T36N, T36M, T36S, T36V. In some of any embodiments, the one or more amino acid substitutions are H19Y/S30G; H19Y/V45A; F14Y/H19Y; H19Y/V45D; H19Y/A43E; H19Y/T36A; H19Y/I22V; N11D/H19Y; H19Y/T36M; N11S/H19Y; H19Y/L35P/T46A; H19Y/N47D; S9D/H19Y; H19Y/S30G/V45D; H19Y/R39Q; H19Y/L35P; S9D/H19Y/R27H; Q10P/H19Y/Q25H; H19Y/R39L/N47D; N11D/H19Y/N47D; H19Y/T32S; N11S/H19Y/S29P; H19Y/R39Q/N47D; S16A/H19Y/R39Q; S9N/H19Y/N31K/T46I; H19Y/R27L/N31Y/T32S/T36A; N11S/H19Y/T46A; H19Y/T32I; S9G/H19Y/T36S/A43S; H19Y/S48G; S9N/H19Y/I22V/N31D; S9N/H19Y/Q25K/N31D; S9G/H19Y/T32S; H19Y/T36A/N47Y; H19Y/V45A/T46I; H19Y/Q25K/N31D; H19Y/Q25H/R39Q/V45D; H19Y/T32S/N47D; Q10E/H19Y/A20T/T36S; H19Y/T32S/V45I; H19F/Q25E/N31L/L35Y/T36S; H19F/Q25F/N31S/T36S; H19I/Q25F/N31S/T36V; H19F/Q25V/N31M/T36S; H19Y/Q25Y/N31L/L35Y/T36S; H19F/Q25I/N31M/L35A/T36S;

H19I/Q25L/N31L/L35Y/T36S; H19F/Q25L/N31G/L35P/T36A; H19Y/I22L/N31G; H19F/I22V/Q25M/N31P/T36M; H19Y/N31L/L35Y/T36S; H19L/S30G/N31H/L35A; H19L/Q25S/N31V/L35S/T36V; H19L/Q25S/S30Y/N31G/L35M/T36V; H19F/Q25F/N31L/L35Y/T36S; H19F/Q25F/N31S/T36G; H19F/I22V/Q25S/N31V/L35S/T36V; H19F/Q25G/N31S/L35V/T36N; H19L/Q25H/N31D/L35S; or H19F/Q25F/N31S/L35Y/T36S.

**[0109]** In some of any embodiments, the one or more amino acid substitutions comprise S16A/H19Y/R39Q. In some of any embodiments, the variant BCMA polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the reference TACI polypeptide.

**[0110]** In some of any embodiments, the variant BCMA polypeptide has increased binding affinity to APRIL. In some of any embodiments, the variant BCMA polypeptide has increased binding affinity to BAFF. In some of any embodiments, the variant BCMA polypeptide has increased binding affinity to APRIL and BAFF.

**[0111]** In some of any embodiments, the increased binding affinity for BAFF or APRIL is independently increased more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold or 60-fold. In some of any embodiments, the variant BCMA polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 357-435. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in any one of SEQ ID NOS: 357-435. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 381. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:411. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:405. In some of any embodiments, the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:406.

**[0112]** In some of any embodiments, the linker comprises a peptide linker and the peptide linker is selected from GSGGS (SEQ ID NO: 592), GGGGS (G4S; SEQ ID NO: 593), GSGGGGS (SEQ ID NO: 590), GGGGSGGGGS (2xGGGGS; SEQ ID NO: 594), GGGGSGGGGSGGGGS (3xGGGGS; SEQ ID NO: 595), GGGGSGGGGSGGGGSGGGGS (4xGGGGS, SEQ ID NO:600), GGGGSGGGGSGGGGSGGGGSGGGGS (5XGGGGS, SEQ ID NO: 671), GGGGSSA (SEQ ID NO: 596) or combinations thereof.

**[0113]** In some of any embodiments, the Fc fusion protein is a dimer. In some of any embodiments, the immunoglobulin Fc region is a homodimeric Fc region.

**[0114]** In some of any embodiments, the immunoglobulin Fc is an IgG1 Fc domain, or is a variant Fc that exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, optionally as compared to a wild-type IgG1 Fc domain. In some embodiments, the immunoglobulin Fc is an IgG1 Fc domain and the Fc includes the amino acid sequence set forth in SEQ ID NO: 597. In some of any

embodiments, the immunoglobulin Fc is a variant IgG1 Fc domain containing one or more amino acid substitutions selected from L234A, L234V, L235A, L235E, G237A, S267K, R292C, N297G, and V302C, by EU numbering. In some of any embodiments, the immunoglobulin Fc region contains the amino acid substitutions L234A, L235E an G237A by EU numbering or the amino acid substitutions R292C, N297G and V302C by EU numbering.

**[0115]** In some of any embodiments, the immunoglobulin Fc is set forth in SEQ ID NO:586. In some embodiments, the Fc is a variant Fc comprising the amino acid sequence set forth in SEQ ID NO:589. In some embodiments, the Fc fusion protein is a homodimer.

**[0116]** In some embodiments, the Fc fusion protein neutralizes APRIL and BAFF. In some embodiments, the IC50 for neutralizing APRIL is less than 100 pM, less than 50 pM, less than 40 pM, less than 30 pM, less than 20 pM, less than 10 pM, less than 5 pM or less than 1 pM, or is any value between any of the foregoing; and/or the IC50 for neutralizing BAFF is less than 400 pM, less than 300 pM, less than 200 pM, less than 100 pM, less than 75 pM, less than 50 pM, less than 25 pm, or less than 10 pM, or is any value between any of the foregoing.

**[0117]** In some of any embodiments, the Fc fusion protein blocks binding of APRIL, BAFF, or an APRIL/BAFF heterotrimer to BCMA or TACI; and the Fc fusion protein reduces the levels of circulating APRIL, BAFF, or an APRIL/BAFF in the blood following administration to a subject. In some of any embodiments, the immunoglobulin Fc is set forth in SEQ ID NO:586. In some of any embodiments, the Fc fusion protein blocks binding of APRIL, BAFF, or an APRIL/BAFF heterotrimer to BCMA or TACI; or the Fc fusion protein reduces the levels of circulating APRIL, BAFF, or an APRIL/BAFF in the blood following administration to a subject. In some of any embodiments, the immunomodulatory protein reduces or inhibits B cell maturation, differentiation and/or proliferation.

**[0118]** Provided herein is a nucleic acid molecule(s) encoding the Fc fusion protein of any of the embodiments described herein. In some of any embodiments, the nucleic acid molecule is a synthetic nucleic acid. In some of any embodiments, the nucleic acid molecule is a cDNA.

**[0119]** Provided herein is a vector containing the nucleic acid molecule of any of the embodiments described herein. In some of any embodiments, the vector is an expression vector. In some of any embodiments, the vector is a mammalian expression vector to a viral vector.

**[0120]** Provided herein is a cell, containing the nucleic acid of any of any of the embodiments described herein or the vector of any of any of any of the embodiments described herein. In some of any embodiments, the cell is a mammalian cell. In some of any embodiments, the cell is a human cell.

**[0121]** Provided herein is a method of producing an immunomodulatory protein, including introducing the nucleic acid molecule of any of any of the embodiments provided herein or vector of any of any of the embodiments provided herein into a host cell under conditions to express the protein in the cell. In some of any embodiments, the method further includes isolating or purifying the Fc fusion

protein from the cell. Provided herein is a method of producing an Fc fusion protein, including introducing the nucleic acid molecule of any of the embodiments provided herein or vector of any of the embodiments provided herein into a host cell under conditions to express the protein in the cell.

**[0122]** Provided herein is an Fc fusion protein produced by the method of any of the embodiments described herein.

**[0123]** Provided herein is a pharmaceutical composition, including the Fc fusion protein of any of any of the embodiments described herein. In some of any embodiments, the pharmaceutical composition contains a pharmaceutically acceptable excipient. In some of any embodiments, the pharmaceutical composition is sterile.

**[0124]** Provided herein is an article of manufacture including the pharmaceutical composition of any of the embodiments described herein in a vial or container. In some of any embodiments, the vial or container is sealed.

**[0125]** Provided herein is a kit including the pharmaceutical composition of any of any of the embodiments provided herein and instructions for use. In some of any embodiments, the kit includes the article of manufacture of any of the embodiments described herein and instructions for use.

**[0126]** Provided herein is a method of reducing an immune response in a subject, including administering the immunomodulatory protein of any of the embodiments described herein to a subject in need thereof.

**[0127]** Provided her~~e~~ein~~e~~ is a method of reducing an immune response in a subject, including administering the Fc fusion protein of any of the embodiments described herein to a subject in need thereof.

**[0128]** Provided herein is a method of reducing an immune response in a subject, including administering the pharmaceutical composition of any of any of the embodiments described herein to a subject in need thereof. In some of any embodiments, a B cell immune response is reduced in the subject, whereby B cell maturation, differentiation and/or proliferation is reduced or inhibited. In some of any embodiments, circulating levels of APRIL, BAFF or an APRIL/BAFF heterotrimer are reduced in the subject.

**[0128a]** Provided herein is a pharmaceutical composition of any of the embodiments described herein for use in reducing an immune response in a subject.

**[0128b]** Provided herein is the use of an immunomodulatory protein or the pharmaceutical composition of any of the embodiments described herein in the manufacture of a medicament for reducing an immune response in a subject.

**[0129]** Provided herein is a method of reducing circulating levels of APRIL, BAFF or an APRIL/BAFF heterotrimer in a subject including administering the pharmaceutical composition of any of any of the embodiments described herein to the subject. In some of any embodiments, a T cell immune

response is reduced in the subject, whereby T cell co-stimulation is reduced or inhibited. In some of any embodiments, reducing the immune response treats a disease or condition in the subject.

**[0129a]** Provided herein is the use of the immunomodulatory protein or the pharmaceutical composition of any of the embodiments described herein in the manufacture of a medicament for reducing circulating levels of APRIL, BAFF, or an APRIL/BAFF heterotrimer in a subject.

**[0130]** Provided herein is a method of treating a disease, disorder or condition in a subject, including administering the immunomodulatory protein of any of any of the embodiments described herein to a subject in need thereof.

**[0131]** Provided herein is a method of treating a disease, disorder or condition in a subject, including administering the Fc fusion protein of any of any of the embodiments described herein to a subject in need thereof.

**[0132]** Provided herein is a method of treating a disease, disorder or condition in a subject, including administering the pharmaceutical composition of any of any of the embodiments described herein to a subject in need thereof.

**[0133]** Also provided herein are any of the immunomodulatory proteins or pharmaceutical compositions containing same for use in treating a disease, disorder or condition in a subject. Also provided herein are uses of any of the immunomodulatory proteins or pharmaceutical compositions containing same for formulation of a medicament for treating a disease, disorder or condition in a subject.

**[0133a]** Provided herein is a pharmaceutical composition of any of the embodiments described herein for use in treating a disease, disorder, or condition in a subject.

**[0133b]** Provided herein is the use of an immunomodulatory protein or the pharmaceutical composition of any of the embodiments described herein in the manufacture of a medicament for treating a disease, disorder, or condition in a subject.

**[0134]** In some of any embodiments, the disease, disorder or condition is an autoimmune disease, an inflammatory condition, a B cell cancer, an antibody- mediated pathology, a renal disease, a graft rejection, graft versus host disease, or a viral infection. The disease or condition that is treated may be any as described herein. In some of any embodiments, the disease or condition is an autoimmune disease selected from the group consisting of Systemic lupus erythematosus (SLE); Sjögren's syndrome, scleroderma, Multiple sclerosis, diabetes, polymyositis, primary biliary cirrhosis, IgA nephropathy, optic neuritis, amyloidosis, antiphospholipid antibody syndrome (APS), autoimmune polyglandular syndrome type II (APS II), autoimmune thyroid disease (AITD), Graves' disease, autoimmune adrenalitis and pemphigus vulgaris. In some of any embodiments, the disease or condition is a B cell cancer and the cancer is myeloma. In some of any embodiments, the type of myeloma includes multiple myeloma, plasmacytoma, multiple solitary plasmacytoma, and/or extramedullary myeloma. In some of any

embodiments, the type of myeloma includes light chain myeloma, nonsecretory myeloma, and/or IgD or IgE myeloma.

Brief Description of the Drawings

**[0135]** **FIG. 1** shows a schematic representation of a functional inhibition assay involving recombinant APRIL and BAFF by BCMA or TACI. In the assay, Jurkat cells transduced with a luciferase-based NF-κB reporter and to stably express mouse or human TACI on the cell-surface expression. Following activation by recombinant APRIL or BAFF, endogenous NF-κB transcription factors bind to the DNA response elements controlling transcription of a firefly luciferase gene. Luciferase expression can be monitored, such as by detection with Bio-Glo™ reagent and measurement using a Cytation 3 reader.

**[0136]** **FIG. 2** shows exemplary human BCMA TD Fc fusion molecules for blockade of human APRIL (top panel) and BAFF (bottom panel) mediated signaling. Exemplary BCMA TD Fc fusions were incubated with APRIL (2nM) or BAFF (4nM) for 20mins (room temperature with shaking) and then added to wells containing 150,000 Jurkat/TACI/NFκB-luciferase cells for 5 hours.

**[0137]** **FIGs. 3A-3B** show function of exemplary BCMA TD Fc fusion molecules alone or when stacked with CTLA-4 IgD for blockade of APRIL (top panel of each FIG) or BAFF (bottom panel of each FIG) . **FIG. 3A** and **FIG. 3B** show human BCMA TDs retain function when stacked with CTLA-4.

**[0138]** **FIGs. 3C-3D** show function of exemplary TACI TD Fc fusion molecules when stacked with CTLA-4 IgD for blockade of APRIL (top panel of each FIG) or BAFF (bottom panel of each FIG) . **FIGs. 3C** and **FIG. 3D** show human TACI TDs retain function when stacked with CTLA-4.

**[0139]** **FIG. 4** shows human BCMA fusion molecules alone or BCMA or TACI TD Fc fusion molecules when stacked with CTLA-4 IgD for blockade of mouse APRIL (left panel) and BAFF (right panel) mediated signaling.

**[0140]** **FIG. 5A** shows human BCMA TD Fc fusion molecules alone or when stacked with CTLA-4 IgD for blockade of human APRIL (top panel) and BAFF (bottom panel) mediated signaling relative to TACI 13-118-Fc , TACI 30-110-Fc, and belimumab.

**[0141]** **FIG. 5B** shows human TACI TD Fc fusion molecules when stacked with CTLA-4 IgD for blockade of human APRIL (tope panel) and BAFF (bottom panel) mediated signaling relative to TACI 13-118-Fc , TACI 30-110-Fc, and belimumab.

**[0142]** **FIG. 6** shows a schematic representation of a functional inhibition assay of CD80/CD86-CD28 mediated costimulation. Jurkat/IL-2 cells stably express a luciferase reporter driven by the IL-2 promoter when stimulated with anti-CD3 and an anti-CD28 stimulus. Receptor-mediated signaling

results in IL-2 promoter-mediated luminescence, and bioluminescent signal can be detected and quantified, such as by using Bio-Glo™ substrate and a luminometer.

**[0143]** **FIG. 7A and FIG. 7B** shows wild type or CTLA-4 vIgDs (IgSF domain) maintain function for blockade of CD80 (left panel) or CD86 (right panel) when included in multi-specific (stack) construct molecules with BCMA to TACI TDs.

**[0144]** **FIGs. 8A-8F** show activity of CTLA-4 vIgD alone or when included in multi-specific (stack) molecules with BCMA or TACI TDs for inhibiting human follicular helper T ($T_{FH}$) and B-cells in an autologous $T_{FH}$-B cell assay. B–Tfh cell cultures were incubated for 7 days in the presence of titrated (100,000 – 32 pM) protein. Cultured cells were surface stained and analyzed by flow cytometry for: (**FIG. 8A**) CD4+ T cell recovery, (**FIG. 8B**) CD4+CD40L+ cell recovery, (**FIG. 8C**) CD4+ICOS+ cell recovery, (**FIG. 8D**) CD19+ B cell recovery, and (**FIG. 8E**) B cell activation/upregulation of CD86. Supernatants were collected and levels of IgM secretion were determined by ELISA (**FIG. 8F**). Data represent an average (± SEM) of three replicates for each condition.

**[0145]** **FIG. 9** shows anti-KLH IgM antibody levels in serum at termination (Day 19) in a KLH immunization model. Statistical differences between each group were determined by 1-way ANOVA; only significant differences ($p < 0.05$) are listed.

**[0146]** **FIG. 10** shows anti-KLH IgG1 antibody levels in serum at termination (Day 19) in a KLH immunization model. Statistical differences between each group were determined by 1-way ANOVA; only significant differences ($p < 0.05$) are listed.

**[0147]** **FIG. 11** shows spleen weight at termination (Day 19) in the KLH model. Statistical differences between the Fc control and other test articles were determined by t-test; only significant ($p < 0.05$) differences are listed.

**[0148]** **FIGs. 12A- 12H** show flow cytometry analyses of splenocyte B cell and Tfh subsets at termination (Day 19) in the KLH model. Spleens were processed and analyzed by flow cytometry for $B220^+$ B cells (**FIG. 12A, FIG. 12E**); Marginal Zone (MZ) B cells (**FIG. 12B, FIG. 12F**); Germinal Center (GC) B cells (**FIG. 12C, FIG. 12G**); T Follicular Helper (Tfh) cells (**FIG. 12D, FIG. 12H**). 'Fc control' = Fc set forth in SEQ ID NO:589. Statistically significant differences ($p < 0.05$) vs. Fc control or abatacept were calculated by 1-way ANOVA with uncorrected Fisher's LSD test.

**[0149]** **FIGs. 13A-13D** show flow cytometry analyses of splenocyte T effector memory subsets at termination (Day 19) in a KLH model. Spleens were processed and analyzed by flow cytometry for $CD4^+$ (**FIG. 13A, FIG. 13C**) and $CD8^+$ (**FIG. 13B, FIG. 13D**) T effector memory ($T_{em}$) cells. 'Fc control' = Fc set forth in SEQ ID NO:589. Statistically significant differences ($p < 0.05$) vs. Fc control or abatacept were calculated by 1-way ANOVA with uncorrected Fisher's LSD.

**[0150]** **FIGs. 14A-14I** show analysis of parameters assessed in an NZB/NZW murine model of human SLE. Proteinuria scores (**FIG.14A**), mean percent change in body weight (**FIG. 14B**), and percent

survival (**FIG. 14C**) were assessed starting at 20 weeks of age. Serum was analyzed for anti-double stranded DNA IgG titers (**FIG. 14D**) and blood urea nitrogen (BUN) (**FIG. 14E**) (** vs Fc by Uncorrected Dunn's test, p=0.0047 and p=0.0065; *** vs Fc by Uncorrected Dunn's test, p=0.0004). Kidneys were processed and analyzed by histology in replicate Periodic acid-Schiff (PAS)-stained sections, with individual component and total histology scores depicted in **FIG. 14F.** Frozen kidneys were also sectioned and stained for immunohistochemical analysis of mouse IgG and complement C3 glomerular deposition, as shown in **FIG. 14G** and **FIG. 14H**, respectively. **FIG. 14I** shows the histological score ±SEM.

**[0151]** **FIG. 15** depicts a schematic representations of an exemplary BCMA-Fc fusion protein.

**[0152]** **FIG. 16** depicts schematic representations of exemplary Fc fusion formats of provided multi-domain (stack) immunomodulatory proteins.

**[0153]** **FIG. 17A and FIG. 17B** depict exemplary sequence alignments to identify corresponding residues in a sequence compared to a reference sequence. The symbol "*" between two aligned amino acid indicates that the aligned amino acids are identical. The symbol "-" indicates a gap in the alignment. Exemplary, non-limiting positions for amino acid substitution described herein are indicated with bold text. Based on the alignment of two similar sequences having identical residues in common, a skilled artisan can identify "corresponding" positions in a sequence by comparison to a reference sequence using conserved and identical amino acid residues as guides. **FIG. 17A** provides an exemplary alignment of a reference TACI extracellular domain sequence set forth in SEQ ID NO:709 (containing the full extracellular domain with a CRD1 and CRD2 and an initiating methionine residue) with a TACI extracellular domain sequence set forth in SEQ ID NO:528 (containing only a single CRD, CRD2); aligning identical residues demonstrates, for example, that amino acid residue E7 in SEQ ID NO:528 corresponds to residue E74 in SEQ ID NO: 709, amino acid residue K10 in SEQ ID NO: 528 corresponds to residue K77 in SEQ ID NO:709, amino acid residue Y12 in SEQ ID NO: 528 corresponds to Y79 in SEQ ID NO:709, amino acid residue L15 in SEQ ID NO:528 corresponds to L82 in SEQ ID NO:709, amino acid residue R17 in SEQ ID NO: 528 corresponds to R84 in SEQ ID NO:709; and amino acid residue D16 in SEQ ID NO:528 correspond to D85 in SEQ ID NO:709. **FIG. 17B** provides an exemplary alignment of a reference BCMA extracellular domain sequence set forth in SEQ ID NO:710 (containing the full extracellular domain with a CRD and an initiating methionine residue) with a BCMA extracellular domain sequence set forth in SEQ ID NO:356 (without the initiating methionine); aligning identical residues demonstrates, for example, that amino acid residue H18 in SEQ ID NO:356 corresponds to residue H19 in SEQ ID NO: 710, and amino acid residue R38 in SEQ ID NO:356 corresponds to residue R39 in SEQ ID NO: 710. It is within the level of a skilled artisan to carry out similar alignments between two similar protein sequences to identify corresponding residues, including based on the exemplification and description herein.

**[0154]** **FIGS. 18A-18D** show analysis of parameters assessed murine keyhole limpet hemocyanin (KLH) model. Serum-KLH IgM OD levels were assessed as primary response (**FIG. 18A)** and secondary response **(FIG. 18B).** Similarly, serum anti-KLH IgG1 OD levels were assessed as both primary response (**FIG. 18C)** and secondary response **(FIG. 18D).**

**[0155]** **FIGS. 19A-19B** shown analysis of harvested spleen assessed from the murine keyhole limpet hemocyanin (KLH) immunization model. Spleens were processed and analyzed by weight (**FIG. 19A**) as well as total cell number (**FIG. 19B**).

**[0156]** **FIG. 20** depicts analysis of spleens assessed for cellular subtype population makeup from the murine keyhole limpet hemocyanin (KLH) model and shows results of B cell subset numbers relative to the group mean.

**[0157]** **FIG. 21** depicts analysis of spleens assessed for cellular subtype phenotype makeup from the murine keyhole limpet hemocyanin (KLH) model and shows results for numbers of germinal center B cells and plasma cells (**FIG. 21**).

**[0158]** **FIGS. 22A-22D** depict T cell numbers in the murine keyhole limpet hemocyanin (KLH) model. The splenic CD3+, CD8+, CD4+ and Follicular Helper T cells are depicted in **FIG. 22A**, **FIG. 22B**, **FIG. 22C**, and **FIG. 22D**, respectively.

**[0159]** **FIG. 23** depicts Tcm and Tem cellular populations in the murine keyhole limpet hemocyanin (KLH) model.

**[0160]** **FIGS. 24A-24B and FIGS. 25A-25B** depict overall incidence and degree of sialadenitis (**FIGS. 24A-24B**) and insulitis (**FIGS. 25A-25B**) in diabetes-prone mice after treatment with 186-CTLA-4 Fc and CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 tested molecules.

**[0161]** **FIG. 26** depicts mean blood glucose concentrations (mg/dL) as measured in the blood on Days 7, 8, 9 and 10 in diabetes-prone mice after treatment with 186-CTLA-4 Fc and CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 tested molecules.

**[0162]** **FIG. 27** and **FIGs. 28A-28C** depict results from an *in vivo* murine bm12 inducible SLE model tested with an exemplary CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 multi-domain molecule, and compared to a WT-TACI Fc only. The BUN concentrations from serum collected at day 82 (study termination) are shown in **FIG. 27. FIGs. 28A-28C** depicts levels of IgG2b (**FIG. 28A**), IgG2c (**FIG. 28B)**, and IgG3 (**FIG. 28C)** from serum collected from days 14, 42 and 82.

**[0163]** **FIG. 29** depicts levels of anti-dsDNA antibody levels from serum collected in an *in vivo* murine bm12 inducible SLE model tested with an exemplary CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 multi-domain molecule.

Detailed Description

**[0164]** Provided herein are immunomodulatory proteins that engage with one or more other immune receptor or ligand, e.g. on antigen-presenting cells or produced as soluble factors, to suppress or reduce B cell responses or activity and, in some cases, also T cell responses. Among the provided immunomodulatory proteins are proteins that bind to BAFF or APRIL ligands to neutralize their activity and block or antagonize the activity of B cell stimulatory receptors, such as TACI or BCMA. The provided immunomodulatory proteins may be fusion proteins of a BCMA extracellular domain or binding portion thereof (hereinafter BCMA ECD) and a multimerization domain, such as an immunoglobulin Fc. For example, provided herein are BCMA-Fc fusion proteins. Still further provided are multi-domain immunomodulatory proteins (also called "stack" immunomodulatory proteins) that contain at least one first binding domain that binds to BAFF or APRIL ligands to neutralize their activity and block or antagonize the activity of B cell stimulatory receptors, such as TACI or BCMA, and at least one second binding domain that blocks or antagonizes the activity of a T cell stimulatory receptor, such as by binding to CD80 or CD86 ligands to neutralize their activity via interactions with the T cell stimulatory receptor CD28 or negative regulatory protein CTLA-4. In some embodiments, the immunomodulatory proteins provided herein can be used for the treatment of diseases, disorders or conditions that are associated with a dysregulated immune response, such as associated with inflammatory or autoimmune symptoms including an inflammatory or autoimmune disease.

**[0165]** The immune system relies on immune checkpoints to prevent autoimmunity (i.e., self-tolerance) and to protect tissues from excessive damage during an immune response, for example during an attack against a pathogenic infection. In some cases, however, the immune system can become dysregulated and an abnormal immune response can be mounted against a normal body part or tissue, resulting in an autoimmune disease or condition or autoimmune symptoms. In other cases an unwanted immune response can be mounted to a foreign tissue, such as a transplant, resulting in transplant rejection.

**[0166]** In some aspects, immunotherapy that alters immune cell activity, such as B cell activity and/or T cell activity, can treat certain diseases, disorders and conditions in which the immune response is dysregulated. In particular, inhibition or attenuation of an immune response, such as a B cell response and/or T cell response, could be desirable to reduce or prevent unwanted inflammation, autoimmune symptoms and/or transplant rejection. Therapeutic approaches that seek to modulate interactions between ligands and their receptors that mediate an immune response, including in the immune synapse, however, are not entirely satisfactory. In some cases, therapies to intervene and alter the immunomodulatory effects of immune cell, e.g. T cell or B cell, activation are constrained by the spatial orientation requirements as well as size limitations imposed by the confines of the immunological synapse. In some aspects, existing therapeutic drugs, including antibody drugs, may not be able to interact simultaneously with the multiple target proteins involved in modulating these interactions. For example, soluble

receptors and antibodies generally bind competitively (e.g., to no more than one target species at a time) and therefore lack the ability to simultaneously bind multiple targets. Additionally, pharmacokinetic differences between drugs that independently target one of these receptors can create difficulties in properly maintaining a desired blood concentration of a drug combination targeting two different targets throughout the course of treatment.

**[0167]** BAFF and APRIL are TNF superfamily members that bind both TACI and BCMA on B cells; BAFF also binds a 3rd receptor, BAFF-R. Together, BAFF and APRIL support B cell development, differentiation, and survival. Their co-neutralization dramatically reduces B cell function, including antibody production, whereas inhibition of either BAFF or APRIL alone mediates relatively modest effects. While CTLA-4-based therapeutics that block T cell costimulation provide safe and moderately effective T cell inhibition in many disease settings, and while B cell targeting therapies have demonstrated promising therapeutic potential, they are not entirely satisfactory.

**[0168]** Among provided embodiments are those that provide for improved neutralizing activity and suppression or reduction of B cell responses. In some embodiments, the improved activity is mediated by increased or improved binding or interaction of the provided immunomodulatory proteins with BAFF and/or APRIL. For example, provided herein are variant BCMA polypeptides that contain one or more amino acid substitutions (replacement or mutations) that confer improved binding affinity of the protein for BAFF and/or APRIL. In particular, among provided embodiments are those that provide for improved, combined BAFF and APRIL inhibition. Further, the provided immunomodulatory proteins include those that suppress BAFF and/or APRIL mediated activity either alone (e.g. BCMA-Fc), or coupled with inhibition of T cell costimulation. For example, among the provided embodiments are multi-domain immunomodulatory protein of a B cell inhibitor molecule (BIM) that is an extracellular domain portion that binds to BAFF and/or APRIL (e.g. a BCMA ECD), fused to a T cell inhibitory molecule (TIM) that is another domain that binds to a ligand of a T cell stimulatory receptor and/or to a T cell stimulatory receptor to antagonize or block T cell responses. It is contemplated that the provided immunomodulatory proteins provide for improved activity to modulate B cell responses alone, or together with modulation of T cell response. Thus, the provided immunomodulatory proteins provide effective and durable disease suppression in the treatment of autoimmune or inflammatory diseases, including in severe B cell-related autoimmune diseases like SLE.

**[0169]** For example, the provided embodiments are based on findings that directed evolution by affinity modification of TNFR domain (TD) of the ectodomain of certain molecules (e.g. BCMA) facilitated the development of molecules with improved affinity for APRIL and/or BAFF. Thus, the affinity modification produces a variant BCMA that contains a variant TNFR domain (vTD). Fusion of such molecules with an immunoglobulin Fc results in immunomodulatory proteins that suppress B cell activity and response. Likewise, the provided embodiments also are based on findings that further

including the TD domains, e.g. wild-type (WT) TD or vTD, as a multi-domain fusion with an immunoglobulin superfamily (IgSF) domain of a T cell inhibitiory molecule, such as a CTLA-4 extracellular domain, further potentiates immunosuppressive activity. Such activity can be further improved by directed evolution by affinity modification of the IgSF domain of CTLA-4 to produce a variant IgSF domain (vIgD) to further enhance affinity for CD80 and/CD86 ligands, which are ligands of the CD28 co-stimulatory receptor and inhibitory CTLA-4 receptor. The findings herein demonstrate these immunomodulatory proteins consistently exhibit potent immunosuppressive activity and efficacy *in vitro* and *in vivo*, appearing superior to existing and/or approved immunomodulators like belimumab, abatacept, atacicept, or telitacicept. Such biologics may therefore be attractive development candidates for the treatment of serious autoimmune and/or inflammatory diseases, including B cell-related diseases such as SLE, Sjögren's syndrome, and other connective tissue diseases.

**[0170]** All publications, including patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

**[0171]** The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

## I. DEFINITIONS

**[0172]** Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

**[0173]** As used in the specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly indicates otherwise.

**[0174]** The term “about” as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*. For example, description referring to “about X” includes description of “X”.

**[0175]** The term “affinity-modified” as used in the context of a domain of a protein means a mammalian protein having an altered amino acid sequence in an extracellular domain or a specific

binding portion thereof (relative to the corresponding wild-type parental or unmodified domain) such that it has an increased or decreased binding activity, such as binding affinity, to at least one of its binding partners (alternatively "counter-structures") compared to the parental wild-type or unmodified (i.e., non-affinity modified domain) protein. In some embodiments, the affinity-modified domain can contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more amino acid differences, such as amino acid substitutions, in a wild-type or unmodified domain. An increase or decrease in binding activity, e.g. binding affinity, can be determined using well known binding assays, including flow cytometry. Larsen et al., American Journal of Transplantation, Vol 5: 443-453 (2005). See also, Linsley et al., Immunity, 1: 7930801 (1994). An increase in a protein's binding activity, e.g. affinity, to its binding partner(s) is to a value at least 10% greater than that of the wild-type control and in some embodiments, at least 20%, 30%, 40%, 50%, 100%, 200%, 300%, 500%, 1000%, 5000%, or 10000% greater than that of the wild-type control value. A decrease in a protein's binding activity, e.g. affinity, to at least one of its binding partner is to a value no greater than 90% of the control but no less than 10% of the wild-type control value, and in some embodiments no greater than 80%, 70% 60%, 50%, 40%, 30%, or 20% but no less than 10% of the wild-type control value. An affinity-modified protein is altered in primary amino acid sequence of the extracellular domain or a specific binding portion thereof by substitution, addition, or deletion of amino acid residues. The term "affinity-modified" is not be construed as imposing any condition for any particular starting composition or method by which the affinity-modified protein was created. Thus, an affinity-modified protein is not limited to wild-type protein domains that are then transformed to an affinity-modified domain by any particular process of affinity modification. An affinity-modified domain polypeptide can, for example, be generated starting from wild-type mammalian domain sequence information, then modeled in silico for binding to its binding partner, and finally recombinantly or chemically synthesized to yield the affinity-modified domain composition of matter. In but one alternative example, an affinity-modified domain can be created by site-directed mutagenesis of a wild-type domain. Thus, affinity modified IgSF domain or an affinity modified TD domain denotes a product and not necessarily a product produced by any given process. A variety of techniques including recombinant methods, chemical synthesis, or combinations thereof, may be employed.

**[0176]** The term "affinity-modified IgSF domain" refers to an affinity-modified domain of a member of the immunoglobulin superfamily (IgSF) protein having an altered amino acid sequence of an immunoglobulin domain (e.g. IgV) within the extracellular domain of the IgSF protein or a specific binding portion thereof (relative to the corresponding wild-type parental or unmodified domain) such that it has an increased or decreased binding activity, such as binding affinity, to at least one of its binding partners (alternatively "counter-structures") compared to the parental wild-type or unmodified protein containing the non-affinity modified or unmodified IgSF domain.

**[0177]** The term "affinity-modified TD domain" refers to an affinity-modified domain of a member of the tumor necrosis receptor superfamily (TNFRSF) protein or a TNF ligand thereof having an altered amino acid sequence of a TNFR domain or of a TNF domain therein, respectively. For example, an affinity-modified TD domain of a TNFRSF protein has an altered amino acid sequence of a TNFR domain composed of at least one cysteine rich domain (CRD) within the extracellular domain of the TNFRSF protein or a specific binding portion thereof (relative to the corresponding wild-type parental or unmodified domain) such that it has an increased or decreased binding activity, such as binding affinity, to at least one of its binding partners (alternatively "counter-structures") compared to the parental wild-type or unmodified protein containing the non-affinity modified or unmodified TD domain.

**[0178]** The term "B cell inhibitory molecule" or BIM refers to a protein molecule that antagonizes or blocks the activity of a B cell stimulatory receptor. The BIM can antagonize the activity of the B cell stimulatory receptor by binding directly to a cognate ligand of the B cell stimulatory receptor, thereby blocking or reducing the binding between the ligand and the B cell stimulatory receptor. For example, a BIM binds to APRIL and/or BAFF, which are ligands of the B cell stimulatory receptors B cell maturation antigen (BCMA), B cell activation factor receptor (BAFF-R), and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI). In particular embodiments, a BIM provided herein contains the extracellular domain or a portion thereof containing a TNF receptor super family domain (TD, e.g. CRD) of a B cell stimulatory receptor that binds to the cognate ligand APRIL and/or BAFF, and heterotrimers of APRIL and BAFF. For example, a BIM includes the extracellular domain of TACI, or a portion of the extracellular domain of TACI containing a TD domain that binds to cognate ligands APRIL and/or BAFF, and heterotrimers of APRIL and BAFF. In other examples, a BIM includes the extracellular domain of BCMA, or a portion of the extracellular domain of BCMA containing a TD domain that binds to cognate ligands APRIL and/or BAFF, and heterotrimers of APRIL and BAFF. A BIM also can include an affinity-modified variant of the extracellular domain or portion thereof of TACI or BCMA with one more amino acid modifications (e.g. amino acid substitutions) in the TD that increase binding affinity for the cognate ligand (e.g. APRIL and/or BAFF, and heterotrimers of APRIL and BAFF).

**[0179]** As used herein, a "B cell stimulatory receptor" refers to one or more of B cell maturation antigen (BCMA), B cell activation factor receptor (BAFF-R), and transmembrane activator and calcium modulatory and cyclophilin ligand interactor (TACI), which are related tumor necrosis factor (TNFR) superfamily receptors expressed on B cells. Engagement or ligation of these related receptors by their cognate ligands, BAFF and/or APRIL, or heterotrimers of APRIL and BAFF, regulate B cell homeostasis, including B cell survival, B cell maturation and differentiation and immunoglobulin class switching. A B cell stimulatory receptor generally contains an extracellular portion, a transmembrane domain and cytoplasmic region, in which the cytoplasmic region contains one or more TNF receptor

associated factor (TRAF) binding sites. Recruitment of various TRAF molecules to the cytoplasmic domain can activate various transcription factors, such as NF-κB (e.g. NF-κB1 or NF-κB2), to mediate B cell signaling pathways regulating B cell homeostasis.

**[0180]** As used herein, "bind," "bound" or grammatical variations thereof refers to the participation of a molecule in any attractive interaction with another molecule, resulting in a stable association in which the two molecules are in close proximity to one another. Binding includes, but is not limited to, non-covalent bonds, covalent bonds (such as reversible and irreversible covalent bonds), and includes interactions between molecules such as, but not limited to, proteins, nucleic acids, carbohydrates, lipids, and small molecules, such as chemical compounds including drugs.

**[0181]** As used herein, binding activity refer to characteristics of a molecule, e.g. a polypeptide, relating to whether or not, and how, it binds one or more binding partners. A binding activity can include any measure of binding of one molecule for a binding partner. Binding activities include the ability to bind the binding partner(s), the affinity with which it binds to the binding partner (e.g. high affinity), the avidity with which it binds to the binding partner, the strength of the bond with the binding partner and/or specificity or selectivity for binding with the binding partner.

**[0182]** The term “binding affinity” as used herein means the specific binding affinity of a protein for its binding partner (i.e., its counter-structure) under specific binding conditions. The binding affinity refers to the strength of the interaction between two or more molecules, such as binding partners, typically the strength of the noncovalent interactions between two binding partners. An increase or attenuation in binding affinity of an affinity-modified domain, or an immunomodulatory protein containing an affinity-modified domain, to a binding partner is determined relative to the binding affinity of the unmodified domain (e.g., the native or wild-type IgSF domain or the native or wild-type TD domain). Methods for determining binding affinity, or relative binding affinity, are known in art, solid-phase ELISA immunoassays, ForteBio Octet, Biacore measurements or flow cytometry. See, for example, Larsen et al., American Journal of Transplantation, vol. 5: 443-453 (2005); Linsley et al., Immunity, Vol 1 (9): 793-801 (1994). In some embodiments, binding affinity can be measured by flow cytometry, such as based on a Mean Fluorescence Intensity (MFI) in a flow binding assay.

**[0183]** The term “binding avidity” as used herein means the specific binding avidity, of a protein for its binding partner (i.e., its counter-structure) under specific binding conditions. In biochemical kinetics avidity refers to the accumulated strength of multiple affinities of individual non-covalent binding interactions, such as between a protein for its binding partner (i.e., its counter-structure). As such, avidity is distinct from affinity, which describes the strength of a single interaction.

**[0184]** The term “biological half-life” refers to the amount of time it takes for a substance, such as an immunomodulatory protein, to lose half of its pharmacologic or physiologic activity or concentration. Biological half-life can be affected by elimination, excretion, degradation (e.g., enzymatic

degradation/digestion) of the substance, or absorption and concentration in certain organs or tissues of the body. In some embodiments, biological half-life can be assessed by determining the time it takes for the blood plasma concentration of the substance to reach half its steady state level ("plasma half-life"). Conjugates that can be used to derivatize and increase the biological half-life of a protein are known in the art and include, but are not limited to, multimerization domains (e.g. Fc immunoglobulin domain), polyethylene glycol (PEG), hydroxyethyl starch (HES), XTEN (extended recombinant peptides; see, WO2013130683), human serum albumin (HSA), bovine serum albumin (BSA), lipids (acylation), and poly-Pro-Ala-Ser (PAS), polyglutamic acid (glutamylation).

**[0185]** The term "cell surface counter-structure" (alternatively "cell surface binding partner") as used herein is a counter-structure (alternatively is a binding partner) expressed on a mammalian cell. Typically, the cell surface binding partner is a transmembrane protein. In some embodiments, the cell surface binding partner is a receptor.

**[0186]** The terms "binding partner" or "counter-structure" in reference to a protein, such as a receptor, soluble ligand, or to an extracellular domain or portion thereof or affinity-modified variant thereof, refers to at least one molecule (typically a native mammalian protein) to which the referenced protein specifically binds under specific binding conditions. In some aspects, an affinity-modified domain, or an immunomodulatory protein containing an affinity-modified domain, specifically binds to the binding partner of the corresponding domain of the native or wild-type protein but with increased or attenuated affinity. A "cell surface binding partner" is a binding partner expressed on a mammalian cell. Typically, the cell surface binding partner is a transmembrane protein. In some embodiments, the cell surface binding partner is a receptor or a ligand of a receptor expressed on and by cells, such as mammalian cells, forming the immunological synapse, for example immune cells.

**[0187]** The term "cis" with reference to binding to cell surface molecules refers to binding to two or more different cell surface molecules, each of which is present on the surface of the same cell. In some embodiments, cis means that the two or more cell surface molecules are exclusively on one or exclusively the other (but not both) of the two mammalian cells forming the IS.

**[0188]** The term "conservative amino acid substitution" as used herein means an amino acid substitution in which an amino acid residue is substituted by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine.

Conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

**[0189]** The term, "corresponding to" with reference to positions of a protein, such as recitation that nucleotides or amino acid positions "correspond to" nucleotides or amino acid positions in a disclosed sequence, such as set forth in the Sequence Listing, refers to nucleotides or amino acid positions identified upon alignment with the disclosed sequence based on structural sequence alignment or using a standard alignment algorithm, such as the GAP algorithm. By aligning the sequences, one skilled in the art can identify corresponding residues, for example, using conserved and identical amino acid residues as guides. **FIG.17A** and **FIG. 17B** exemplify identification of corresponding residues by aligning two sequences.

**[0190]** As used herein, "domain" (typically a sequence of three or more, generally 5 or 7 or more amino acids, such as 10 to 200 amino acid residues) refers to a portion of a molecule, such as a protein or encoding nucleic acid, that is structurally and/or functionally distinct from other portions of the molecule and is identifiable. For example, domains include those portions of a polypeptide chain that can form an independently folded structure within a protein made up of one or more structural motifs and/or that is recognized by virtue of a functional activity, such as binding activity. A protein can have one, or more than one, distinct domains. For example, a domain can be identified, defined or distinguished by homology of the primary sequence or structure to related family members, such as homology to motifs. In another example, a domain can be distinguished by its function, such as an ability to interact with a biomolecule, such as a cognate binding partner. A domain independently can exhibit a biological function or activity such that the domain independently or fused to another molecule can perform an activity, such as, for example binding. A domain can be a linear sequence of amino acids or a non-linear sequence of amino acids. Many polypeptides contain a plurality of domains. Such domains are known, and can be identified by those of skill in the art. For exemplification herein, definitions are provided, but it is understood that it is well within the skill in the art to recognize particular domains by name. If needed appropriate software can be employed to identify domains. It is understood that reference to amino acids, including to a specific sequence set forth as a SEQ ID NO used to describe domain organization (e.g. of an IgSF domain or a TD domain) are for illustrative purposes and are not meant to limit the scope of the embodiments provided. It is understood that polypeptides and the description of domains thereof are theoretically derived based on homology analysis and alignments with similar molecules. Also, in some cases, adjacent N- and/or C-terminal amino acids of a given domain (e.g. IgSF domain or TD) also can be included in a sequence, such as to ensure proper folding of the domain when expressed. Thus, the exact locus can vary, and is not necessarily the same for each protein. For example, a specific IgSF domain, such as specific IgV domain or IgC domain, can be several amino acids (1-10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids) longer or shorter. Likewise, a specific TD domain, such

as specific CRD domain, can be several amino acids (1-10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids) longer or shorter.

**[0191]** The term "ectodomain," "extracellular domain," or "ECD," which are used interchangeably herein, refers to a region of a membrane protein, such as a transmembrane protein, that lies outside the vesicular membrane (e.g., the space outside of a cell), when a full-length form of the membrane protein is expressed from a cell. For purposes herein, it is understood that reference to the ECD refers to sequences and domains that make up this region and do not require that a protein that contains an ECD is a membrane protein or that the domain is present outside a cell. For example, a soluble immunomodulatory protein can contain ECD sequences of a membrane protein fused to another moiety, such as a multimerization domain, for example an Fc region. Ectodomains often interact with specific ligands or specific cell surface receptors, such as via a binding domain that specifically binds to the ligand or cell surface receptor. Examples of binding domains include immunoglobulin domains (IgD, also called an IgSF domain) or cysteine rich domains (CRDs). Ectodomains of members of the immunoglobulin superfamily contain an IgD (e.g. IgV domain). Ectodomains of members of the TNFR superfamily contain a TD domain (e.g. a CRD domain). Thus reference to an ECD herein includes a full-length sequence of an ECD of a membrane protein as well as specific-binding fragments thereof containing an IgD or a CRD that bind to a ligand or cognate binding partner.

**[0192]** The terms "effective amount" or "therapeutically effective amount" refer to a quantity and/or concentration of a therapeutic composition, such as containing an immunomodulatory protein or Fc fusion protein, that when administered ex vivo (by contact with a cell from a patient) or in vivo (by administration into a patient) either alone (i.e., as a monotherapy) or in combination with additional therapeutic agents, yields a statistically significant inhibition of disease progression as, for example, by ameliorating or eliminating symptoms and/or the cause of the disease. An effective amount for treating a disease, condition or disorder, such as an immune system disease or disorder, may be an amount that relieves, lessens, or alleviates at least one symptom or biological response or effect associated with the disease, condition or disorder, prevents progression of the disease, condition or disorder, or improves physical functioning of the patient. In the case of cell therapy, the effective amount is an effective dose or number of cells administered to a patient. In some embodiments the patient is a human patient.

**[0193]** As used herein, a fusion protein refers to a polypeptide encoded by a nucleic acid sequence containing a coding sequence for two or more proteins, in some cases 2, 3, 4, 5 or more protein, in which the coding sequences are in the same reading frame such that when the fusion construct is transcribed and translated in a host cell, the protein is produced containing the two or more proteins. Each of the two or more proteins can be adjacent to another protein in the construct or separated by a linker polypeptide that contains, 1, 2, 3, or more, but typically fewer than 20, 15, 10, 9, 8, 7, or 6 amino acids. The protein product encoded by a fusion construct is referred to as a fusion polypeptide. An example of a fusion

protein in accord with the provided embodiments is an Fc fusion protein containing an affinity-modified domain (e.g. a variant of a BCMA or portion thereof containing a CRD) that is linked to an immunoglobulin Fc domain.

**[0194]** The term "half-life extending moiety" refers to a moiety of a polypeptide fusion or chemical conjugate that extends the half-life of a protein circulating in mammalian blood serum compared to the half-life of the protein that is not so conjugated to the moiety. In some embodiments, half-life is extended by greater than or about 1.2-fold, about 1.5-fold, about 2.0-fold, about 3.0-fold, about 4.0-fold, about 5.0-fold, or about 6.0-fold. In some embodiments, half-life is extended by more than 6 hours, more than 12 hours, more than 24 hours, more than 48 hours, more than 72 hours, more than 96 hours or more than 1 week after *in vivo* administration compared to the protein without the half-life extending moiety. The half-life refers to the amount of time it takes for the protein to lose half of its concentration, amount, or activity. Half-life can be determined for example, by using an ELISA assay or an activity assay. Exemplary half-life extending moieties include an Fc domain, a multimerization domain, polyethylene glycol (PEG), hydroxyethyl starch (HES), XTEN (extended recombinant peptides; see, WO2013130683), human serum albumin (HSA), bovine serum albumin (BSA), lipids (acylation), and poly-Pro-Ala-Ser (PAS), and polyglutamic acid (glutamylation).

**[0195]** An Fc (fragment crystallizable) region or domain of an immunoglobulin molecule (also termed an Fc polypeptide) corresponds largely to the constant region of the immunoglobulin heavy chain, and which, in some cases, is responsible for various functions, including the antibody's effector function(s). The Fc domain contains part or all of a hinge domain of an immunoglobulin molecule plus a CH2 and a CH3 domain. In some cases for inclusion in a provided fusion protein, all or a portion of the Fc hinge sequence may be deleted. The Fc domain can form a dimer of two polypeptide chains joined by one or more disulfide bonds. In some embodiments, the Fc is a variant Fc that exhibits reduced (e.g. reduced greater than about 30%, 40%, 50%, 60%, 70%, 80%, 90% or more) activity to facilitate an effector function. In some embodiments, reference to amino acid substitutions in an Fc region is by EU numbering system unless described with reference to a specific SEQ ID NO. EU numbering is known and is according to the most recently updated IMGT Scientific Chart (IMGT®, the international ImMunoGeneTics information system®, http://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html (created: 17 May 2001, last updated: 10 Jan 2013) and the EU index as reported in Kabat, E.A. et al. Sequences of Proteins of Immunological interest. 5th ed. US Department of Health and Human Services, NIH publication No. 91-3242 (1991).

**[0196]** An immunoglobulin Fc fusion ("Fc-fusion"), such as an immunomodulatory Fc fusion protein, is a molecule comprising one or more polypeptides operably linked to an Fc region of an immunoglobulin. An Fc-fusion may comprise, for example, an Fc region operably linked to a TIM or

BIM of the provided immunomodulatory proteins. An Fc-fusion may comprise, for example, an Fc region operably linked to a BCMA extracellular domain or portion thereof containing a CRD, including any of the provided affinity-modified variants thereof. An immunoglobulin Fc region may be linked indirectly or directly to the one or more polypeptides. Various linkers are known in the art and can optionally be used to link an Fc to a fusion partner to generate an Fc-fusion. Fc-fusions of identical species can be dimerized to form Fc-fusion homodimers. Fc fusion of non-identical species (e.g. knob into hole engineering) may be used to form Fc-fusion heterodimers. In some embodiments, the Fc is a mammalian Fc such as a murine or human Fc.

**[0197]** The term "host cell" refers to any cell that can be used to express a protein encoded by a recombinant expression vector. A host cell can be a prokaryote, for example, E. coli, or it can be a eukaryote, for example, a single-celled eukaryote (e.g., a yeast or other fungus), a plant cell (e.g., a tobacco or tomato plant cell), an animal cell (e.g., a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Examples of host cells include Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media or CHO strain DX-B11, which is deficient in DHFR.

**[0198]** The term "immunological synapse" or "immune synapse" (abbreviated "IS") as used herein means the interface between a mammalian cell that expresses MHC I (major histocompatibility complex) or MHC II, such as an antigen-presenting cell or tumor cell, and a mammalian lymphocyte such as an effector T cell or Natural Killer (NK) cell.

**[0199]** The term "immunoglobulin" (abbreviated "Ig") as used herein is synonymous with the term "antibody" (abbreviated "Ab") and refers to a mammalian immunoglobulin protein including any of the five human classes: IgA (which includes subclasses IgA1 and IgA2), IgD, IgE, IgG (which includes subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. The term is also inclusive of immunoglobulins that are less than full-length, whether wholly or partially synthetic (e.g., recombinant or chemical synthesis) or naturally produced, including any fragment thereof containing at least a portion of the variable heavy (VH) chain and/or variable light (VL) chain region of the immunoglobulin molecule that is sufficient to form an antigen binding site and, when assembled, to specifically bind antigen. The antibody also can include all or a portion of the constant region. Such fragments include antigen binding fragment (Fab), variable fragment (Fv) containing VH and VL, the single chain variable fragment (scFv) containing VH and VL linked together in one chain, as well as other antibody V region fragments, such as Fab′, F(ab)2, F(ab′)2, dsFv diabody, Fc, and Fd polypeptide fragments. Hence, it is understood that reference to an antibody herein includes full-length antibody and antigen-binding fragments. The term antibody also includes antibody compositions with polyepitopic specificity, multispecific antibodies (*e.g.*, bispecific antibodies), diabodies, and single-chain molecules. Bispecific antibodies, homobispecific and heterobispecific, are included within the meaning of the term. Antibodies include polyclonal antibodies

or monoclonal antibodies. Antibody also includes synthetic antibodies or recombinantly produced antibodies. For the structure and properties of the different classes of antibodies, see *e.g.*, *Basic and Clinical Immunology*, 8th Edition, Daniel P. Sties, Abba I. Terr and Tristram G. Parsolw (eds), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

**[0200]** The terms "full-length antibody," "intact antibody" or "whole antibody" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. A full-length antibody is an antibody typically having two full-length heavy chains (*e.g.*, VH-CH1-CH2-CH3 or VH-CH1-CH2-CH3-CH4) and two full-length light chains (VL-CL) and hinge regions, such as antibodies produced from mammalian species (e.g. human, mouse, rat, rabbit, non-human primate, etc.) by antibody secreting B cells and antibodies with the same domains that are produced synthetically. Specifically whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

**[0201]** An "antibody fragment" comprises a portion of an intact antibody, the antigen binding and/or the variable region of the intact antibody. Antibody fragments, include, but are not limited to, Fab fragments, Fab' fragments, $F(ab')_2$ fragments, Fv fragments, disulfide-linked Fvs (dsFv), Fd fragments, Fd' fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata *et al., Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules, including single-chain Fvs (scFv) or single-chain Fabs (scFab); antigen-binding fragments of any of the above and multispecific antibodies from antibody fragments.

**[0202]** "Fv" is composed of one heavy- and one light-chain variable region domain linked by non-covalent association. From the folding of these two domains emanate six complementarity determining regions (CDR) (3 in each from the heavy and light chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although, in some cases, at a lower affinity than the entire binding site.

**[0203]** "dsFv" refers to an Fv with an engineered intermolecular disulfide bond, which stabilizes the $V_H$-$V_L$ pair.

**[0204]** An "Fd fragment" is a fragment of an antibody containing a variable domain ($V_H$) and one constant region domain ($C_H1$) of an antibody heavy chain.

**[0205]** A "Fab fragment" is an antibody fragment that results from digestion of a full-length immunoglobulin with papain, or a fragment having the same structure that is produced synthetically, *e.g.,* by recombinant methods. A Fab fragment contains a light chain (containing a $V_L$ and $C_L$) and another

chain containing a variable domain of a heavy chain ($V_H$) and one constant region domain of the heavy chain ($C_H1$).

**[0206]** A "F(ab')$_2$ fragment" is an antibody fragment that results from digestion of an immunoglobulin with pepsin at pH 4.0-4.5, or a fragment having the same structure that is produced synthetically, *e.g.,* by recombinant methods. The F(ab')$_2$ fragment essentially contains two Fab fragments where each heavy chain portion contains an additional few amino acids, including cysteine residues that form disulfide linkages joining the two fragments.

**[0207]** A "Fab' fragment" is a fragment containing one half (one heavy chain and one light chain) of the F(ab')$_2$ fragment.

**[0208]** An "Fd' fragment" is a fragment of an antibody containing one heavy chain portion of a F(ab')$_2$ fragment.

**[0209]** An "Fv' fragment" is a fragment containing only the $V_H$ and $V_L$ domains of an antibody molecule.

**[0210]** An "scFv fragment" refers to an antibody fragment that contains a variable light chain ($V_L$) and variable heavy chain ($V_H$), covalently connected by a polypeptide linker in any order. The linker is of a length such that the two variable domains are bridged without substantial interference. Exemplary linkers are $(Gly\text{-}Ser)_n$ residues with some Glu or Lys residues dispersed throughout to increase solubility.

**[0211]** "Diabodies" are dimeric scFv; diabodies typically have shorter peptide linkers than scFvs, and preferentially dimerize.

**[0212]** The term "immunoglobulin superfamily" or "IgSF" as used herein means the group of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. Molecules are categorized as members of this superfamily based on shared structural features with immunoglobulins (i.e., antibodies); they all possess a domain known as an immunoglobulin domain or fold. Many "non-antibody IgSF" members include cell surface proteins or receptors that are not antibodies. Members of the IgSF include cell surface antigen receptors, co-receptors and co-stimulatory molecules of the immune system, molecules involved in antigen presentation to lymphocytes, cell adhesion molecules, certain cytokine receptors and intracellular muscle proteins. They are commonly associated with roles in the immune system. Proteins in the immunological synapse are often members of the IgSF. IgSF can also be classified into "subfamilies" based on shared properties such as function. Such subfamilies typically include from 4 to 30 IgSF members.

**[0213]** The terms "IgSF domain" or "immunoglobulin domain" or "Ig domain" or "IgD" as used herein refers to a structural domain or domains of IgSF proteins. Ig domains are named after the immunoglobulin molecules. They contain about 70-110 amino acids and are categorized according to their size and function. Ig-domains possess a characteristic Ig-fold, which has a sandwich-like structure formed by two sheets of antiparallel beta strands. Interactions between hydrophobic amino acids on the

inner side of the sandwich and highly conserved disulfide bonds formed between cysteine residues in the B and F strands, stabilize the Ig-fold. In some cases, one end of the Ig domain has a section called the complementarity determining region, which, in some aspects, is involved in the specificity of antibodies for their ligands. The Ig like domains can be classified (into classes) as: IgV, IgC1, IgC2, or IgI. Most Ig domains are either variable (IgV) or constant (IgC). IgV domains with 9 beta strands are generally longer than IgC domains with 7 beta strands. Ig domains of some members of the IgSF resemble IgV domains in the amino acid sequence, yet are similar in size to IgC domains. These are called IgC2 domains, while standard IgC domains are called IgC1 domains. T-cell receptor (TCR) chains contain two Ig domains in the extracellular portion; one IgV domain at the N-terminus and one IgC1 domain adjacent to the cell membrane. A "non-antibody IgSF domain" refers to IgSF domain or domains present in proteins other than antibodies, which typically are present in the extracellular portion or domain of certain cell surface proteins. Thus, the extracellular domain (ECD) of IgSF family members contains one or more Ig domains; hence, the term Ig domain is also used with reference to the ECD of such protein molecules. Reference to a variant IgSF domain (vIgD) refers to a variant or modified sequence of an IgD.

**[0214]** The term "immunological activity" as used herein refers to one or more activities of immune cells, such as T cells or B cells, including, for example, activation, cell survival, cell proliferation, cytokine production (e.g. interferon-gamma), cytotoxicity activity, or ability to activate NF-κB pathway or other signaling cascade leading to activation of a transcription factor in the immune cell. Assays to assess immunological activity of immunomodulatory proteins can be compared to control proteins with a known activity.

**[0215]** An "immunomodulatory protein" or "immunomodulatory polypeptide" is a protein that modulates immunological activity. By "modulation" or "modulating" an immune response is meant that immunological activity is either enhanced or suppressed. Such modulation includes any induction, or alteration in degree or extent, or suppression of immunological activity of an immune cell, such as a B cell or a T cell. For example, soluble Fc fusion proteins herein may suppress immunological activity of either B cells, T cells or both B cells and T cells. An immunomodulatory protein can be a single polypeptide chain or a multimer (dimers or higher order multimers) of at least two polypeptide chains covalently bonded to each other by, for example, interchain disulfide bonds. Thus, monomeric, dimeric, and higher order multimeric proteins are within the scope of the defined term. Multimeric proteins can be homomultimeric (of identical polypeptide chains) or heteromultimeric (of different polypeptide chains).

**[0216]** As used herein, modification is in reference to modification of a sequence of amino acids of a polypeptide or a sequence of nucleotides in a nucleic acid molecule and includes a change in amino acids or nucleotides, respectively, of the sequence. The amino acid modification or change may be a

deletion, insertion, or replacement (substitution) of amino acids or nucleotides, respectively. Methods of modifying a polypeptide are routine to those of skill in the art, such as by using recombinant DNA methodologies.

**[0217]** The term, a "multimerization domain" refers to a sequence of amino acids that promotes the formation of a multimer of two or more polypeptides. A multimerization domain includes sequences that promote stable interaction of a polypeptide molecule with one or more additional polypeptide molecules, each containing a complementary multimerization domain (e.g. a first multimerization domain and a second multimerization domain), which can be the same or a different multimerization domain. The interactions between complementary multimerization domains, e.g. interaction between a first multimerization domain and a second multimerization domain, form a stable protein-protein interaction to produce a multimer of the polypeptide molecule with the additional polypeptide molecule. In some cases, the multimerization domain is the same and interacts with itself to form a stable protein-protein interaction between two polypeptide chains. Generally, a polypeptide is joined directly or indirectly to the multimerization domain. Exemplary multimerization domains include the immunoglobulin sequences or portions thereof, leucine zippers, hydrophobic regions, hydrophilic regions, and compatible protein-protein interaction domains. The multimerization domain, for example, can be an immunoglobulin constant region or domain, such as, for example, the Fc domain or portions thereof from IgG, including IgG1, IgG2, IgG3 or IgG4 subtypes, IgA, IgE, IgD and IgM and modified forms thereof.

**[0218]** The terms "nucleic acid" and "polynucleotide" are used interchangeably to refer to a polymer of nucleic acid residues (e.g., deoxyribonucleotides or ribonucleotides) in either single- or double-stranded form. Unless specifically limited, the terms encompass nucleic acids containing known analogues of natural nucleotides and that have similar binding properties to it 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 nucleotide sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may 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. The term nucleic acid or polynucleotide encompasses cDNA or mRNA encoded by a gene.

**[0219]** The terms "in operable combination," "in operable order" and "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner or orientation that the segments are arranged so that they function in concert for their intended purposes. In some embodiments, the term refers to linkage of nucleic acids to produce a nucleic acid molecule capable of directing the transcription of a given gene and/or to produce a desired protein molecule that is functional. For example, segments of a DNA sequence, e.g. a coding sequence and a regulatory sequence(s), are linked in such a way as to

permit gene expression when the appropriate molecules (e.g. transcriptional activator proteins) are bound to the regulatory sequence.

**[0220]** The term "pharmaceutical composition" refers to a composition suitable for pharmaceutical use in a mammalian subject, often a human. A pharmaceutical composition typically comprises an effective amount of an active agent (e.g., an immunomodulatory protein) and a carrier, excipient, or diluent. The carrier, excipient, or diluent is typically a pharmaceutically acceptable carrier, excipient or diluent, respectively.

**[0221]** The terms "polypeptide" and "protein" are used interchangeably herein and refer to a molecular chain of two or more amino acids linked through peptide bonds. The terms do not refer to a specific length of the product. Thus, "peptides," and "oligopeptides," are included within the definition of polypeptide. The terms include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. The terms also include molecules in which one or more amino acid analogs or non-canonical or unnatural amino acids are included as can be synthesized, or expressed recombinantly using known protein engineering techniques. In addition, proteins can be derivatized as described herein by well-known organic chemistry techniques.

**[0222]** The term "purified" as applied to nucleic acids, such as encoding immunomodulatory proteins, or proteins (e.g. immunomodulatory proteins) generally denotes a nucleic acid or polypeptide that is substantially free from other components as determined by analytical techniques well known in the art (e.g., a purified polypeptide or polynucleotide forms a discrete band in an electrophoretic gel, chromatographic eluate, and/or a media subjected to density gradient centrifugation). For example, a nucleic acid or polypeptide that gives rise to essentially one band in an electrophoretic gel is "purified." A purified nucleic acid or protein is at least about 50% pure, usually at least about 75%, 80%, 85%, 90%, 95%, 96%, 99% or more pure (e.g., percent by weight or on a molar basis).

**[0223]** The term "recombinant" indicates that the material (e.g., a nucleic acid or a polypeptide) has been artificially (i.e., non-naturally) altered by human intervention. The alteration can be performed on the material within, or removed from, its natural environment or state. For example, a "recombinant nucleic acid" is one that is made by recombining nucleic acids, e.g., during cloning, affinity modification, DNA shuffling or other well-known molecular biological procedures. A "recombinant DNA molecule," is comprised of segments of DNA joined together by means of such molecular biological techniques. The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule (e.g., an immunomodulatory protein) which is expressed using a recombinant DNA molecule. A "recombinant host cell" is a cell that contains and/or expresses a recombinant nucleic acid or that is otherwise altered by genetic engineering, such as by introducing into the cell a nucleic acid molecule encoding a recombinant protein, such as a immunomodulatory protein provided herein. Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers

consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription. Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect and mammalian cells and viruses (analogous control elements, i.e., promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the protein of interest.

**[0224]** The term "recombinant expression vector" as used herein refers to a DNA molecule containing a desired coding sequence (e.g., encoding an immunomodulatory protein) and appropriate nucleic acid sequences necessary for the expression of an operably linked coding sequence in a particular cell. Nucleic acid sequences necessary for expression in prokaryotes include a promoter, optionally an operator sequence, a ribosome binding site and possibly other sequences. Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals. A secretory signal peptide sequence can also, optionally, be encoded by the recombinant expression vector, operably linked to the coding sequence so that the expressed protein can be secreted by the recombinant host cell, such as for its expression as a secretable protein or for more facile isolation or purification of the immunomodulatory protein from the cell, if desired. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Among the vectors are viral vectors, such as lentiviral vectors.

**[0225]** The term "sequence identity" as used herein refers to the sequence identity between genes or proteins at the nucleotide or amino acid level, respectively. "Sequence identity" is a measure of identity between proteins at the amino acid level and a measure of identity between nucleic acids at nucleotide level. The protein sequence identity may be determined by comparing the amino acid sequence in a given position in each sequence when the sequences are aligned. Similarly, the nucleic acid sequence identity may be determined by comparing the nucleotide sequence in a given position in each sequence when the sequences are aligned. Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software, FASTA and TFASTA. The BLAST algorithm calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Center for Biotechnology Information (NCBI) website. In some cases, a percent sequence identity can be determined as the percentage of amino acid residues (or nucleotide residues) in a candidate sequence that are identical with the amino acid residues (or nucleotide residues) in a reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Reference to sequence identity includes sequence identity across the full length of each of the sequences being compared. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

**[0226]** The term "soluble" as used herein in reference to proteins means that the protein is not a membrane protein or is not anchored in a cell membrane. A protein can be constructed as a soluble protein by inclusion of only an extracellular domain or a portion thereof and without a transmembrane domain. In some cases, solubility of a protein can be improved by linkage or attachment, directly or indirectly via a linker, to an Fc domain or other half-life extending molecule, which, in some cases, also can improve the stability and/or half-life of the protein. In some aspects, a soluble protein is an Fc fusion protein.

**[0227]** The term "specifically binds" as used herein means the ability of a protein, under specific binding conditions, to bind to a target protein such that its affinity or avidity is at least 10 times as great, but optionally 50, 100, 250 or 500 times as great, or even at least 1000 times as great as the average affinity or avidity of the same protein to a collection of random peptides or polypeptides of sufficient statistical size. A specifically binding protein need not bind exclusively to a single target molecule but may specifically bind to more than one target molecule. In some cases, a specifically binding protein may bind to a protein that has similarity in structural conformation with the target protein (e.g., paralogs or orthologs). Those of skill will recognize that specific binding to a molecule having the same function in a different species of animal (i.e., ortholog) or to a molecule having a substantially similar epitope as the target molecule (e.g., paralog) is possible and does not detract from the specificity of binding which is determined relative to a statistically valid collection of unique non-targets (e.g., random polypeptides). Thus, an immunomodulatory protein of the invention, or BIM or TIM thereof, may specifically bind to more than one distinct species of target molecule due to cross-reactivity. Solid-phase ELISA immunoassays, ForteBio Octet or Biacore measurements can be used to determine specific binding between two proteins. Generally, interactions between two binding proteins have dissociation constants (Kd) less than about $1x10^{-5}$ M, and often as low as about 1 x $10^{-12}$ M. In certain aspects of the present disclosure, interactions between two binding proteins have dissociation constants of less than about $1x10^{-6}$ M, $1x10^{-7}$ M, $1x10^{-8}$ M, $1x10^{-9}$ M, $1x10^{-10}$ M, or $1x10^{-11}$ M or less.

**[0228]** The term "specific binding fragment" or "fragment" as used herein in reference to a protein means a polypeptide that is shorter than a full-length protein or a specific domain or region thereof and that specifically binds in vitro and/or in vivo to a binding partner of the full-length protein or of the specific domain or region. A specific finding fragment is in reference to a fragment of a full length extracellular domain of a polypeptide or a binding domain of a polypeptide, but that still binds to a binding partner of the binding domain. For example, a specific binding fragment is in reference to a fragment of a full-length extracellular domain of an IgSF family member or a full-length IgSF domain thereof (e.g. IgV or IgC), but that still binds to a binding partner of the IgSF family member or of an IgSF domain of an IgSF family member. In another examples, a specific binding fragment is in reference to a fragment of an extracellular domain of a full-length TNFR family member or a full-length TNFR domain

(TD) thereof (e.g. CRD), but that still binds to a binding partner of the TNFR family member or of a CRD of an TNFR family member. In some embodiments, the specific binding fragment is at least about 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% the sequence length of the full-length sequence of the extracellular domain or of a domain or region of the extracellular domain. In some embodiments, the specific binding fragment can have an amino acid length of at least 50 amino acids, such as at least 60, 70, 80, 90, 100, or 110 amino acids.

**[0229]** As used herein, a "subject" is a mammal, such as a human or other animal, and typically is human. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects.

**[0230]** As used herein, "synthetic," with reference to, for example, a synthetic nucleic acid molecule or a synthetic gene or a synthetic peptide refers to a nucleic acid molecule or polypeptide molecule that is produced by recombinant methods and/or by chemical synthesis methods.

**[0231]** The term "TNF receptor superfamily" or "TNFRSF" as used herein means the group of cell surface cytokine receptors that are all type I (N-terminus extracellular) transmembrane glycoproteins that contain one to six cysteine rich domains (CRD) in their extracellular domain. Molecules are categorized as members of this superfamily based on the shared structural features that include the one or more cysteine rich domain (CRD) present in their N-terminal extracellular region, which often play a role in protein binding of their cognate binding partner or ligand. A TNFRSF protein may have only one or several CRDs (e.g. CRD1, CRD2, etc.). Typically, ECD or ectodomain of TNFRSF members contain between 1 and 6 pseudorepeats of CRDs. For example, BAFF-receptor and BCMA each contain one CRD while TACI contains two CRDs (CRD1 and CRD2). TNFRSF members are usually trimeric or multimeric complexes that are stabilized by their intracysteine disulfide bonds. Binding of TNFRSF proteins to their ligands facilitates various biological activities in cells, such as the induction of apoptotic cell death or cell survival and proliferation.

**[0232]** The term "TD" refers to a structural domain or domains of TNFRSF proteins or of TNF family ligands. For example, a TD of a TNFRSF protein is a cysteine-rich domain (CRD) module of about 40 amino acids containing six (6) conserved cysteines. Hence, reference to CRD also can be used interchangeably with the term TD in reference to a TD of a TNFRSF protein. The six cysteines are involved in formation of intrachain disulphide bonds. The extracellular domain (ECD) of TNFRSF members contains one or more CRD domains; hence, the term TD is also used with reference to the ECD of such protein molecules. Reference to a variant TD (vTD) refers to a variant or modified sequence of a TD.

**[0233]** The term "T cell inhibitory molecule" or TIM refers to a protein molecule that antagonizes or blocks the activity of a T cell stimulatory receptor. The TIM can antagonize the activity of the T cell stimulatory receptor by binding directly to the T cell stimulatory receptor or a ligand of the T cell

stimulatory receptor, thereby blocking or reducing the binding between the ligand and the T cell stimulatory receptor. For example, a TIM antagonizes or inhibits activity of a T cell costimulatory receptor, such as CD28. In particular embodiments, a TIM provided herein contains the extracellular domain or a portion thereof containing an immunoglobulin superfamily (IgSF) domain, such as an IgV domain, of a cognate ligand of a T cell stimulatory receptor. For example, a TIM includes the extracellular domain of CTLA-4, or a portion of the extracellular domain of CTLA-4 containing an IgSF domain (e.g. IgV domain) that binds to a T cell stimulatory receptor (e.g. CD28). A TIM also can include an affinity-modified variant of the extracellular domain or portion thereof of a cognate ligand of the T cell stimulatory receptor, e.g. CTLA-4, with one more amino acid modifications (e.g. amino acid substitutions) in the IgSF domain that increase binding affinity for the T cell stimulatory receptor (e.g. CD28).

**[0234]** As used herein, a "T cell stimulatory receptor" refers to a cell surface molecule expressed on a T cell in which engagement or ligation of the molecule results in the direct or indirect activation of one or more tyrosine kinases in the cell and/or culminates in the induction or potentiation of one or more effector cell functions the T cell in which it is expressed. A T cell stimulatory receptor generally contains an extracellular portion, a transmembrane domain and cytoplasmic region. In some embodiments, the cytoplasmic region contains an intracellular signaling domain that contains an immunoreceptor tyrosine-based activation motif (ITAM; defined by the sequence YXX(L/I)X6–8YXX(L/I)) or that otherwise is capable of interacting with or associating with one or more accessory proteins, such as one or more adaptor proteins, involved in or regulating tyrosine phosphorylation in a signal transduction pathway. In some cases, a T cell stimulatory receptor interacts with or associates with an adaptor protein that contains an ITAM or an adaptor protein that contains one or more protein-binding domains, such as e.g., Src homology 2 (SH2) and SH3 domains, that bind specific amino acid sequences, e.g phosphotyrosine residues, within a protein in a signal transduction pathway. Examples of adaptor proteins include, but are not limited to, Lck, Fyn, ZAP70, SLP76, PI3K, Grb2, PKCϴ and SHC1. Thus, it is understood that the T cell stimulatory receptor itself need not possess intrinsic enzymatic activity but may indirectly mediate enzymatic activities via accessory or adaptor proteins. Typically, engagement of a T cell stimulatory receptor initiates, mediates. or potentiates activation of the T cell resulting in a measurable morphological, phenotypic, and/or functional changes in the T cell, including cell proliferation, cytolytic activity, cytokine production or secretion, or expression of cell surface molecules such as receptors or adhesion molecules. In some embodiments, T cell stimulatory receptor includes a T cell receptor (TCR), CD3, CD4, CD8, CD28, ICOS, or CD2. For example, the T cell stimulatory receptor is a costimulatory receptor, such as CD28.

**[0235]** The term "trans" with reference to binding to cell surface molecules refers to binding to two different cell surface molecules, each of which is present on the surface of a different cell. In some

embodiments, trans means that with respect to two different cell surface molecules, the first is exclusively present on one of the two mammalian cells forming the IS and the second is present exclusively on the second of the two mammalian cells forming the IS.

**[0236]** The term "transmembrane protein" as used herein means a membrane protein that substantially or completely spans a lipid bilayer such as those lipid bilayers found in a biological membrane such as a mammalian cell, or in an artificial construct such as a liposome. The transmembrane protein comprises a transmembrane domain ("transmembrane domain") by which it is integrated into the lipid bilayer and by which the integration is thermodynamically stable under physiological conditions. Transmembrane domains are generally predictable from their amino acid sequence via any number of commercially available bioinformatics software applications on the basis of their elevated hydrophobicity relative to regions of the protein that interact with aqueous environments (e.g., cytosol, extracellular fluid). A transmembrane domain is often a hydrophobic alpha helix that spans the membrane. A transmembrane protein can pass through the both layers of the lipid bilayer once or multiple times.

**[0237]** The terms "treating," "treatment," or "therapy" of a disease, condition or disorder as used herein mean slowing, stopping or reversing the disease or disorders progression, as evidenced by decreasing, cessation or elimination of either clinical or diagnostic symptoms, by administration of an immunomodulatory protein or engineered cells of the present invention either alone or in combination with another compound as described herein. "Treating," "treatment," or "therapy" also means a decrease in the severity of symptoms in an acute or chronic disease, condition or disorder or a decrease in the relapse rate as for example in the case of a relapsing or remitting autoimmune disease course or inflammatory condition or a decrease in inflammation in the case of an inflammatory aspect of an autoimmune disease or inflammatory condition. "Preventing," "prophylaxis," or "prevention" of a disease or disorder as used in the context of this invention refers to the administration of an immunomodulatory protein, either alone or in combination with another compound, to prevent the occurrence or onset of a disease or disorder or some or all of the symptoms of a disease, condition or disorder or to lessen the likelihood of the onset of a disease, condition or disorder.

**[0238]** The term "variant" (also "modified" or mutant," which can be used interchangeably) as used in reference to a variant protein or polypeptide means a protein, such as a mammalian (e.g., human or murine) protein created by human intervention. The variant is a polypeptide having an altered or modified amino acid sequence, such as by one or more amino acid substitutions, deletions, additions or combinations thereof, relative to an unmodified or wild-type protein or to a domain thereof. A variant polypeptide can contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more amino acid differences, such as amino acid substitutions. A variant polypeptide generally exhibits at least 50%, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to a corresponding form of a wild-type or unmodified protein, such as a mature sequence thereof (lacking the signal sequence) or a portion thereof containing the extracellular domain or an binding domain thereof. Non-naturally occurring amino acids as well as naturally occurring amino acids are included within the scope of permissible substitutions or additions. A variant protein is not limited to any particular method of making and includes, for example, chemical synthesis, recombinant DNA techniques, or combinations thereof. A variant protein of the invention specifically binds to at least one or more binding partners. In some embodiments, the altered amino acid sequence results in an altered (i.e., increased or decreased) binding activity, such as binding affinity or avidity, to the one or more binding partners. A variant protein may thus be an "affinity-modified" protein as described herein.

**[0239]** The term "wild-type" or "natural" or "native," which are used interchangeably, as used herein is used in connection with biological materials such as nucleic acid molecules, proteins, host cells, and the like, that are found in nature and not modified by human intervention.

## II. BCMA IMMUNOMODULATORY PROTEINS AND VARIANT BCMA POLYPEPTIDES

**[0240]** Provided herein are BCMA immunomodulatory proteins that contain a portion of the extracellular domain (ECD) of the BCMA receptor, or a variant thereof, that bind to at least one BCMA cognate binding partner. Also provided herein are variant BCMA polypeptides that exhibit altered (e.g. increased) binding activity or affinity for one or more of a BCMA cognate binding partner. In some embodiments, the BCMA cognate binding partner is one or more of BAFF or APRIL or is a BAFF/APRIL heterotrimer. The provided BCMA immunomodulatory proteins and polypeptides include soluble fusion proteins thereof in which the BCMA portion of the extracellular domain or variant thereof is linked to another moiety, such as an immunoglobulin Fc or other multimerization domain or half-life extending moiety. Thus, in some embodiments the immunomodulatory protein is a BCMA-Fc fusion protein. In some embodiments, provided is a BCMA-Fc fusion protein containing (1) a BCMA polypeptide composed of the extracellular domain of the BCMA receptor or a portion thereof, or a variant BCMA polypeptide thereof , that binds to at least one BCMA cognate binding partner, and (2) an Fc domain. The BCMA polypeptide or variant BCMA polypeptide can be linked directly or indirectly (e.g. via a peptide linker) to the Fc domain.

**[0241]** BCMA is a tumor necrosis factor receptor family member characterized by having an extracellular domain (ECD) containing cysteine-rich pseudo-repeat domain (CRD). BCMA is a membrane bound receptor, which has an extracellular domain containing a single CRD, a transmembrane domain and a cytoplasmic domain that contains TRAF-binding sites for binding to TRAF signaling molecules. BCMA binds to cognate ligands APRIL and BAFF, although binding to BAFF is with weaker affinity. It is reported that BCMA binds to BAFF with two to three orders of magnitude weaker

binding than binding between BAFF and its other cognate receptors BAFF-R and TACI (Bossen and Schneider et al. 2006 Seminars in Immunology, 18:263-75).

**[0242]** The amino acid sequence of the full length BCMA is set forth in SEQ ID NO:667. The protein is a type II membrane protein and lacks a signal peptide; following expression in eukaryotic cells the N-terminal methionine is removed. In some embodiments, a mature BCMA protein does not contain the N-terminal methionine as set forth in SEQ ID NO:667. The extracellular domain of BCMA (amino acid residues 1-54 of SEQ ID NO:667; ECD set forth in SEQ ID NO:710) contains one cysteine rich domain (CRD, hereinafter also called a tumor necrosis family receptor domain or TD), which exhibits affinity for binding to APRIL and to a lesser extent BAFF. The CRD contains amino acid residues 7-41 of the sequence set forth in SEQ ID NO:710.

**[0243]** In some embodiments, the variant BCMA polypeptides provided herein contain one or more amino acid modifications, such as one or more substitutions (alternatively, "mutations" or "replacements"), deletions or additions in the extracellular domain of a reference BCMA polypeptide, such as a wild-type or unmodified BCMA polypeptide containing a CRD (hereinafter also called TD). Thus, a provided variant BCMA polypeptide is or comprises a variant TD ("vTD") in which the one or more amino acid modifications (e.g. substitutions) is in the CRD.

**[0244]** In some embodiments, the reference (e.g. unmodified) BCMA sequence is a wild-type BCMA sequence or is a portion thereof that contains the CRD. In some embodiments, the reference (e.g., unmodified) BCMA is or comprises the extracellular domain (ECD) of BCMA or a portion thereof containing the CRD. In some embodiments, the variant BCMA polypeptide comprises or consists essentially of the CRD or a specific binding fragment thereof. In some embodiments, the variant BCMA is a soluble polypeptide and lacks a transmembrane domain. In some embodiments, the variant BCMA polypeptide further comprises a transmembrane domain and, in some cases, also a cytoplasmic domain.

**[0245]** In some embodiments, the reference (e.g., unmodified) BCMA sequence is a mammalian BCMA sequence. In some embodiments, the reference (e.g., unmodified) BCMA sequence can be a mammalian BCMA that includes, but is not limited to, human, mouse, cynomolgus monkey, or rat. In some embodiments, the reference (e.g., unmodified) BCMA sequence is human. The extracellular domain of an exemplary human BCMA sequence is set forth in SEQ ID NO:710.

**[0246]** In some embodiments, the reference (e.g., unmodified) BCMA sequence has (i) the sequence of amino acids set forth in SEQ ID NO:710 or a sequence thereof that lacks the N-terminal methionine, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:710 and that binds to APRIL or BAFF, or (iii) is a fragment or portion of (i) or (ii) containing a CRD, in which the portion binds to APRIL or BAFF. In some embodiments, the reference (e.g., unmodified) BCMA sequence lacks the N-terminal methionine as set forth in SEQ ID NO: 710.

**BCMA Extracellular Domain (ECD): SEQ ID NO:710**
MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVKGTNA

**[0247]** In some embodiments, the reference (e.g., unmodified) BCMA sequence lacks the N-terminal methionine as set forth in SEQ ID NO: 710. In some embodiments, the reference (e.g., unmodified) BCMA sequence has (i) the sequence of amino acids set forth in SEQ ID NO:356, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:356 and that binds to APRIL or BAFF, or (iii) is a fragment or portion of (i) or (ii) containing a CRD, in which the portion binds to APRIL or BAFF.

**BCMA Extracellular Domain (ECD): SEQ ID NO:356**
LQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVKGTNA

**[0248]** Among provided BCMA polypeptides are variant BCMA polypeptides. Also provided are immunomodulatory proteins, such as BCMA-Fc fusion proteins, that contain a provided variant BCMA polypeptide. In some of any of the provided embodiments, the variant BCMA sequence has the sequence of the reference (e.g. unmodified) BCMA sequence, such as any described above, but additionally contains one more amino acid modifications, such as one or more amino acid substitutions. In particular, provided herein are variant BCMA polypeptides containing at least one affinity-modified TD domain (CRD) or a specific binding fragment thereof that contains one or more amino acid substitutions in a TD domain of a reference (e.g., unmodified or wild-type) BCMA polypeptide, such that the variant BCMA polypeptide exhibits altered (e.g. increased) binding activity or affinity for one or both of APRIL or BAFF compared to the reference (e.g., unmodified or wild-type) BCMA polypeptide. In some embodiments, a variant BCMA polypeptide has a binding affinity for APRIL and/or BAFF that differs from that of a reference (e.g., unmodified or wild-type) BCMA polypeptide control sequence as determined by, for example, solid-phase ELISA immunoassays, flow cytometry or Biacore assays. Binding affinities for each of the cognate binding partners are independent; that is, in some embodiments, a variant BCMA polypeptide has an increased binding affinity for one or both APRIL and BAFF, and a decreased or unchanged binding affinity for the other of APRIL or BAFF, relative to a reference (e.g., unmodified or wild-type) BCMA polypeptide.

**[0249]** In some embodiments, the variant BCMA polypeptide has an increased binding affinity for BAFF, relative to the reference (unmodified or wild-type) BCMA polypeptide. In some embodiments, the variant BCMA polypeptide has an increased binding affinity for APRIL relative to the reference

(unmodified or wild-type) BCMA polypeptide. In some embodiments, the variant BCMA polypeptide has an increased binding affinity for APRIL and BAFF relative to the reference (unmodified or wild-type) BCMA polypeptide. The cognate ligands BAFFF and/or APRIL can be a mammalian protein, such as a human protein or a murine protein. In some embodiments, a variant BCMA polypeptide with increased or greater binding affinity to APRIL and/or BAFF will have an increase in binding affinity relative to the reference (e.g., unmodified or wild-type) BCMA polypeptide control of at least about 5%, such as at least about 10%, 15%, 20%, 25%, 35%, or 50%. In some embodiments, the increase in binding affinity relative to the reference (e.g., unmodified or wild-type) BCMA polypeptide is more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold 40-fold or 50-fold. In any of the examples, the reference (e.g., unmodified or wild-type) BCMA polypeptide has the same sequence as the variant BCMA polypeptide except that it does not contain the one or more amino acid modifications (e.g., substitutions).

**[0250]** In some embodiments, the equilibrium dissociation constant ($K_d$) of any of the foregoing embodiments to BAFF can be less than $1x10^{-5}$ M, $1x10^{-6}$ M, $1x10^{-7}$ M, $1x10^{-8}$ M, $1x10^{-9}$ M, $1x10^{-10}$ M or $1x10^{-11}$M, or $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to BAFF is less than at or about $1x10^{-9}$ M, $1x10^{-10}$ M or $1x10^{-11}$M, or $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to BAFF is between $1x10^{-9}$ M and at or about $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to BAFF is at or about $1x10^{-9}$ M, at or about $2x10^{-9}$ M, at or about $4x10^{-9}$ M, at or about $6x10^{-9}$ M, at or about $8x10^{-9}$ M, at or about $1x10^{-10}$ M, at or about $2x10^{-10}$ M, at or about $4x10^{-10}$ M, at or about $6x10^{-10}$ M, at or about $8x10^{-10}$ M, at or about $1x10^{-11}$ M, at or about $2x10^{-11}$ M, at or about $4x10^{-11}$ M, at or about $6x10^{-11}$ M, at or about $8x10^{-11}$ M, or at or about $1x10^{-12}$ M, or any value between any of the foregoing. In some embodiments, a provided embodiment includes a variant BCMA polypeptide as described above and the $K_d$ to BAFF is decreased (higher binding affinity) by greater than or greater than about 1.5-fold, such as greater than or about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold or more.

**[0251]** In some embodiments, the equilibrium dissociation constant ($K_d$) of any of the foregoing embodiments to APRIL can be less than $1x10^{-5}$ M, $1x10^{-6}$ M, $1x10^{-7}$ M, $1x10^{-8}$ M, $1x10^{-9}$ M, $1x10^{-10}$ M or $1x10^{-11}$M, or $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to APRIL is less than at or about $1x10^{-9}$ M, $1x10^{-10}$ M or $1x10^{-11}$M, or $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to APRIL is between $1x10^{-9}$ M and at or about $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to APRIL is at or about $1x10^{-9}$ M, at or about $2x10^{-9}$ M, at or about $4x10^{-9}$ M, at or about $6x10^{-9}$ M, at or about $8x10^{-9}$ M, at or about $1x10^{-10}$ M, at or about $2x10^{-10}$ M, at or about $4x10^{-10}$ M, at or about $6x10^{-10}$ M, at or about $8x10^{-10}$ M, at or about $1x10^{-11}$ M, at or about $2x10^{-11}$ M, at or about $4x10^{-11}$ M, at or about $6x10^{-11}$ M, at or about $8x10^{-11}$ M, or at or about $1x10^{-12}$ M, or any value between any of the foregoing. In some embodiments, a provided

embodiment includes a variant BCMA polypeptide as described above and the $K_d$ to APRIL is decreased (higher binding affinity) by greater than or greater than about 1.5-fold, such as greater than or about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold or more.

**[0252]** The reference (e.g., unmodified or wild-type) BCMA sequence does not necessarily have to be used as a starting composition to generate variant BCMA polypeptides described herein. Therefore, use of the term "modification", such as "substitution" does not imply that the present embodiments are limited to a particular method of making variant BCMA polypeptides or immunomodulatory proteins containing the same. Variant BCMA polypeptides can be made, for example, by *de novo* peptide synthesis and thus does not necessarily require a modification, such as a "substitution", in the sense of altering a codon to encode for the modification, e.g. substitution. This principle also extends to the terms "addition" and "deletion" of an amino acid residue which likewise do not imply a particular method of making. The means by which the variant BCMA polypeptides are designed or created is not limited to any particular method. In some embodiments, however, a reference (e.g., unmodified or wild-type) BCMA encoding nucleic acid is mutagenized from reference (e.g., unmodified or wild-type) BCMA genetic material and screened for desired specific binding affinity or other functional activity. In some embodiments, a variant BCMA polypeptide is synthesized *de novo* utilizing protein or nucleic acid sequences available at any number of publicly available databases and then subsequently screened. The National Center for Biotechnology Information provides such information and its website is publicly accessible via the internet as is the UniProtKB database as discussed previously.

**[0253]** Unless stated otherwise, as indicated throughout the present disclosure, the amino acid modification (s) in a variant BCMA polypeptide are designated by amino acid position number corresponding to the numbering of positions of the reference ECD sequence set forth in SEQ ID NO:710. It is within the level of a skilled artisan to identify the corresponding position of a modification, e.g. amino acid substitution, in an BCMA polypeptide, including portion thereof containing TD (CRD) thereof, such as by alignment of a reference sequence (e.g. SEQ ID NO:356) with SEQ ID NO:710. An alignment identifying corresponding residues is exemplified in **FIG. 17B.** In the listing of modifications throughout this disclosure, the amino acid position is indicated in the middle, with the corresponding reference (e.g. unmodified or wild-type) amino acid listed before the number and the identified variant amino acid substitution listed after the number. If the modification is a deletion of the position a "del" is indicated and if the modification is an insertion at the position an "ins" is indicated. In some cases, an insertion is listed with the amino acid position indicated in the middle, with the corresponding reference amino acid listed before and after the number and the identified variant amino acid insertion listed after the unmodified (e.g. wild-type) amino acid.

**[0254]** In some embodiments, the variant BCMA polypeptide has one or more amino acid modification, e.g. substitution in a reference (e.g., unmodified or wild-type) BCMA sequence, such as

any as described. The one or more amino acid modification, e.g. substitution, can be in the ectodomain (extracellular domain) of the reference (e.g., unmodified or wild-type) BCMA sequence. In some embodiments, the one or more amino acid modification, e.g. substitution is in the CRD domain or specific binding fragment thereof.

**[0255]** In some embodiments, the variant BCMA polypeptide has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modification(s), e.g. substitution. The modification, e.g. substitution can be in the CRD. In some embodiments, the variant BCMA polypeptide has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions in the CRD or specific binding fragment thereof. In some embodiments, the variant BCMA polypeptide containing the one or more amino acid modifications (e.g. amino acid substitutions) as described has at least about 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the reference (e.g., unmodified or wild-type) BCMA polypeptide or specific binding fragment thereof, such as with the amino acid sequence of SEQ ID NO: 710 or 356. In some embodiments, the variant BCMA polypeptide containing the one or more amino acid modifications (e.g. amino acid substitutions) as described has at least about 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 710. In some embodiments, the variant BCMA polypeptide containing the one or more amino acid modifications (e.g. amino acid substitutions) as described has at least about 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 356.

**[0256]** In some embodiments, the variant BCMA polypeptide has one or more amino acid modification, e.g. substitution in a reference BCMA polypeptide or specific binding fragment there of corresponding to position(s) 9, 10, 11, 14, 16, 19, 20, 22, 25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 or 48 with reference to numbering of SEQ ID NO:710. In some embodiments, the variant BCMA polypeptide has one or more amino acid modification, e.g. substitution selected from S9G, S9N, S9Y, Q10E, Q10P, N11D, N11S, F14Y, S16A, H19A, H19C, H19D, H19E, H19F, H19G, H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R, H19S, H19T, H19V, H19W, H19Y, A20T, I22L, I22V, Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y, R27H, R27L, S29P, S30G, S30Y, N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y, T32I, T32S, L35A, L35M, L35P, L35S, L35V, L35Y, T36A, T36G, T36N, T36M, T36S, T36V, R39L, R39Q, A43E, A43S, V45A, V45D, V45I, T46A, T46I, N47D, N47Y, S48G, or a conservative amino acid substitution thereof. In some embodiments, the reference BCMA polypeptide is set forth in SEQ ID NO: 356.

**[0257]** A conservative amino acid modification, e.g. substitution is any amino acid that falls in the same class of amino acids as the substituted amino acids, other than the reference (e.g., unmodified) or wild-type amino acid. The classes of amino acids are aliphatic (glycine, alanine, valine, leucine, and

isoleucine), hydroxyl or sulfur-containing (serine, cysteine, threonine, and methionine), cyclic (proline), aromatic (phenylalanine, tyrosine, tryptophan), basic (histidine, lysine, and arginine), and acidic/amide (aspartate, glutamate, asparagine, and glutamine).

**[0258]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 9. In some embodiment, the at least one amino acid substitution is S9G, S9N, S9Y.

**[0259]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 10. In some embodiment, the at least one amino acid substitution is Q10E, Q10P.

**[0260]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 11. In some embodiment, the at least one amino acid substitution is N11D, N11S.

**[0261]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 19. In some embodiment, the at least one amino acid substitution is H19A, H19C, H19D, H19E, H19F, H19G, H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R, H19S, H19T, H19V, H19W, H19Y. In some embodiments, the at least one amino acid substitution is H19L. In some embodiments, the at least one amino acid substitution is H19K. In some embodiments, the at least one amino acid substitution is H19Q. In some embodiments, the at least one amino acid substitution is H19R. In some embodiments, the at least one amino acid substitution is H19Y.

**[0262]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 22. In some embodiment, the at least one amino acid substitution is I22L, I22V.

**[0263]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 25. In some embodiment, the at least one amino acid substitution is Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y.

**[0264]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 27. In some embodiment, the at least one amino acid substitution is R27H, R27L.

**[0265]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 30. In some embodiment, the at least one amino acid substitution is S30G, S30Y.

**[0266]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 31. In some embodiment, the at least one amino acid substitution is N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y.

**[0267]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 32. In some embodiment, the at least one amino acid substitution is T32I, T32S.

**[0268]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 35. In some embodiment, the at least one amino acid substitution is L35A, L35M, L35P, L35S, L35V, L35Y.

**[0269]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 36. In some embodiment, the at least one amino acid substitution is T36A, T36G, T36N, T36M, T36S, T36V.

**[0270]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 39. In some embodiment, the at least one amino acid substitution is R39L, R39Q.

**[0271]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 43. In some embodiment, the at least one amino acid substitution is A43E, A43S.

**[0272]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 45. In some embodiment, the at least one amino acid substitution is V45A, V45D, V45I.

**[0273]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 46. In some embodiment, the at least one amino acid substitution is T46A, T46I.

**[0274]** In some embodiments, the variant BCMA polypeptide includes at least one amino acid substitution at position 47. In some embodiment, the at least one amino acid substitution is N47D, N47Y.

**[0275]** In some embodiments, the one or more amino acid substitutions comprise S16A/H19Y/R39Q.

**[0276]** In some embodiments, the variant BCMA polypeptide comprises any of the mutations listed in Table 1. Table 1 also provides exemplary sequences by reference to SEQ ID NO of the reference (e.g., unmodified) BCMA polypeptide, and exemplary variant BCMA polypeptides. As indicated, the exact locus or residues corresponding to a given domain can vary, such as depending on the methods used to identify or classify the domain. Also, in some cases, adjacent N- and/or C-terminal amino acids of a given domain (e.g. CRD) also can be included in a sequence of a variant BCMA polypeptide, such as to ensure proper folding of the domain when expressed. Thus, it is understood that the exemplification of the SEQ ID NOSs in Table 1 is not to be construed as limiting. For example, the particular domain, such as the ECD domain or a portion thereof containing the CRD1/CRD2 or CRD2 only, of a variant BCMA polypeptide can be several amino acids longer or shorter, such as 1-10, e.g., 1, 2, 3, 4, 5, 6 or 7 amino acids longer or shorter, than the sequence of amino acids set forth in the respective SEQ ID NO.

**[0277]** In some embodiments, the variant BCMA polypeptide comprises any of the mutations listed in Table 1. In some examples, the mutations are made in a reference BCMA containing the sequence of amino acids set forth in SEQ ID NO: 710. In some examples, the mutations are made in a reference BCMA containing the sequence of amino acids set forth in SEQ ID NO: 356.

**[0278]** The use of the term “modification”, such as “substitution” or “mutation,” does not imply that the present embodiments are limited to a particular method of making the immunomodulatory proteins. A variant BCMA polypeptide can be made, for example, by *de novo* peptide synthesis and thus

does not necessarily require a modification, such as a "substitution" in the sense of altering a codon to encode for the modification, e.g. substitution. This principle also extends to the terms "addition" and "deletion" of an amino acid residue which likewise do not imply a particular method of making. The means by which the vTDs are designed or created is not limited to any particular method. In some embodiments, however, a wild-type or unmodified TD encoding nucleic acid is mutagenized from wild-type or unmodified TD genetic material and screened for desired specific binding activity, e.g. binding affinity, and/or alteration of NF-κB modulation or other functional activity. In some embodiments, a vTD is synthesized *de novo* utilizing protein or nucleic acid sequences available at any number of publicly available databases and then subsequently screened. The National Center for Biotechnology Information provides such information and its website is publicly accessible via the internet as is the UniProtKB database.

**[0279]** In some embodiments, the variant BCMA polypeptide comprises an extracellular domain (ECD) sequences set forth in any one of SEQ ID NOS: 357-435. In some embodiments, the variant BCMA polypeptide comprises a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 357-435, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) BCMA. In some embodiments, the variant BCMA polypeptide comprises a specific binding fragment of any one of SEQ ID NOS: 357-435, in which the specific binding fragment binds BAFF and/or APRIL and contains a contiguous sequence therein that contains the amino acid modification(s), e.g. substitution (s) therein not present in the reference (e.g., unmodified or wild-type) BCMA.

**[0280]** In some embodiments, the variant BCMA polypeptide comprises the sequence set forth in SEQ ID NO:381. In some embodiments, the variant BCMA polypeptide consists essentially of the sequence set forth in SEQ ID NO:381. In some embodiments, the variant BCMA polypeptide consists of the sequence set forth in SEQ ID NO:381.

**[0281]** In some embodiments, the variant BCMA polypeptide comprises the sequence set forth in SEQ ID NO:405. In some embodiments, the variant BCMA polypeptide consists essentially of the sequence set forth in SEQ ID NO:405. In some embodiments, the variant BCMA polypeptide consists of the sequence set forth in SEQ ID NO:405.

**[0282]** In some embodiments, the variant BCMA polypeptide comprises the sequence set forth in SEQ ID NO:406. In some embodiments, the variant BCMA polypeptide consists essentially of the sequence set forth in SEQ ID NO:406. In some embodiments, the variant BCMA polypeptide consists of the sequence set forth in SEQ ID NO:406.

**[0283]** In some embodiments, the variant BCMA polypeptide comprises the sequence set forth in SEQ ID NO:410. In some embodiments, the variant BCMA polypeptide consists essentially of the sequence set forth in SEQ ID NO:410. In some embodiments, the variant BCMA polypeptide consists of the sequence set forth in SEQ ID NO:410.

**[0284]** In some embodiments, the variant BCMA polypeptide comprises the sequence set forth in SEQ ID NO:411. In some embodiments, the variant BCMA polypeptide consists essentially of the sequence set forth in SEQ ID NO:411. In some embodiments, the variant BCMA polypeptide consists of the sequence set forth in SEQ ID NO:411.

**[0285]** In some embodiments, the variant BCMA polypeptide is encoded by a sequence of nucleotides set forth in any of SEQ ID NOS: 437-515. In some embodiments, the variant BCMA polypeptide is encoded by a sequence of nucleotides that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 437-515, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) BCMA. Also provided herein is a nucleic acid containing the sequence set forth in any of SEQ ID NOS: 437-515 or a sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 437-515.

**TABLE 1: Exemplary variant BCMA in BCMA immunomodulatory protein (e.g. BCMA-Fc) or as a BIM**

| Name | Mutation(s) | ECD SEQ ID NO | |
|---|---|---|---|
| | | AA SEQ ID NO | NT SEQ ID NO |
| 356 (WT) | Wild-type | 356 | 436 |
| 357 BCMA | H19Y | 357 | 437 |
| 358 BCMA | H19Y, S30G | 358 | 438 |
| 359 BCMA | H19Y, V45A | 359 | 439 |
| 360 BCMA | F14Y, H19Y | 360 | 440 |
| 361 BCMA | H19Y, V45D | 361 | 441 |
| 362 BCMA | H19Y, A43E | 362 | 442 |
| 363 BCMA | H19Y, T36A | 363 | 443 |
| 364 BCMA | H19Y, I22V | 364 | 444 |
| 365 BCMA | N11D, H19Y | 365 | 445 |
| 366 BCMA | H19Y, T36M | 366 | 446 |
| 367 BCMA | N11S, H19Y | 367 | 447 |
| 368 BCMA | H19Y, L35P, T46A | 368 | 448 |
| 369 BCMA | H19Y, N47D | 369 | 449 |

**TABLE 1: Exemplary variant BCMA in BCMA immunomodulatory protein (e.g. BCMA-Fc) or as a BIM**

| Name | Mutation(s) | ECD SEQ ID NO | |
|---|---|---|---|
| | | AA SEQ ID NO | NT SEQ ID NO |
| 370 BCMA | S9D, H19Y | 370 | 450 |
| 371 BCMA | H19Y, S30G, V45D | 371 | 451 |
| 372 BCMA | H19Y, R39Q | 372 | 452 |
| 373 BCMA | H19Y, L35P | 373 | 453 |
| 374 BCMA | S9D, H19Y, R27H | 374 | 454 |
| 375 BCMA | Q10P, H19Y, Q25H | 375 | 455 |
| 376 BCMA | H19Y, R39L, N47D | 376 | 456 |
| 377 BCMA | N11D, H19Y, N47D | 377 | 457 |
| 378 BCMA | H19Y, T32S | 378 | 458 |
| 379 BCMA | N11S, H19Y, S29P | 379 | 459 |
| 380 BCMA | H19Y, R39Q, N47D | 380 | 460 |
| 381 BCMA | S16A, H19Y, R39Q | 381 | 461 |
| 382 BCMA | S9N, H19Y, N31K, T46I | 382 | 462 |
| 383 BCMA | H19Y, R27L, N31Y, T32S, T36A | 383 | 463 |
| 384 BCMA | N11S, H19Y, T46A | 384 | 464 |
| 385 BCMA | H19Y, T32I | 385 | 465 |
| 386 BCMA | S9G, H19Y, T36S, A43S | 386 | 466 |
| 387 BCMA | H19Y, S48G | 387 | 467 |
| 388 BCMA | S9N, H19Y, I22V, N31D | 388 | 468 |
| 389 BCMA | S9N, H19Y, Q25K, N31D | 389 | 469 |
| 390 BCMA | S9G, H19Y, T32S | 390 | 470 |
| 391 BCMA | H19Y, T36A, N47Y | 391 | 471 |
| 392 BCMA | H19Y, V45A, T46I | 392 | 472 |
| 393 BCMA | H19Y, Q25K, N31D | 393 | 473 |
| 394 BCMA | H19Y, Q25H, R39Q, V45D | 394 | 474 |
| 395 BCMA | H19Y, T32S, N47D | 395 | 475 |
| 396 BCMA | Q10E, H19Y, A20T, T36S | 396 | 476 |
| 397 BCMA | H19Y, T32S, V45I | 397 | 477 |
| 398 BCMA | H19A | 398 | 478 |
| 399 BCMA | H19C | 399 | 479 |
| 400 BCMA | H19D | 400 | 480 |
| 401 BCMA | H19E | 401 | 481 |
| 402 BCMA | H19F | 402 | 482 |
| 403 BCMA | H19G | 403 | 483 |
| 404 BCMA | H19I | 404 | 484 |
| 405 BCMA | H19K | 405 | 485 |
| 406 BCMA | H19L | 406 | 486 |
| 407 BCMA | H19M | 407 | 487 |
| 408 BCMA | H19N | 408 | 488 |

| TABLE 1: Exemplary variant BCMA in BCMA immunomodulatory protein (e.g. BCMA-Fc) or as a BIM | | | |
|---|---|---|---|
| **Name** | **Mutation(s)** | **ECD SEQ ID NO** | |
| | | **AA SEQ ID NO** | **NT SEQ ID NO** |
| 409 BCMA | H19P | 409 | 489 |
| 410 BCMA | H19Q | 410 | 490 |
| 411 BCMA | H19R | 411 | 491 |
| 412 BCMA | H19S | 412 | 492 |
| 413 BCMA | H19T | 413 | 493 |
| 414 BCMA | H19V | 414 | 494 |
| 415 BCMA | H19W | 415 | 495 |
| 416 BCMA | H19F, Q25E, N31L, L35Y, T36S | 416 | 496 |
| 417 BCMA | H19F, Q25F, N31S, T36S | 417 | 497 |
| 418 BCMA | H19I, Q25F, N31S, T36V | 418 | 498 |
| 419 BCMA | H19F, Q25V, N31M, T36S | 419 | 499 |
| 420 BCMA | H19Y, Q25Y, N31L, L35Y, T36S | 420 | 500 |
| 421 BCMA | H19F, Q25I, N31M, L35A, T36S | 421 | 501 |
| 422 BCMA | H19I, Q25L, N31L, L35Y, T36S | 422 | 502 |
| 423 BCMA | H19F, Q25L, N31G, L35P, T36A | 423 | 503 |
| 424 BCMA | H19Y, I22L, N31G | 424 | 504 |
| 425 BCMA | H19F, I22V, Q25M, N31P, T36M | 425 | 505 |
| 426 BCMA | H19Y, N31L, L35Y, T36S | 426 | 506 |
| 427 BCMA | H19L, S30G, N31H, L35A | 427 | 507 |
| 428 BCMA | H19L, Q25S, N31V, L35S, T36V | 428 | 508 |
| 429 BCMA | H19L, Q25S, S30Y, N31G, L35M, T36V | 429 | 509 |
| 430 BCMA | H19F, Q25F, N31L, L35Y, T36S | 430 | 510 |
| 431 BCMA | H19F, Q25F, N31S, T36G | 431 | 511 |
| 432 BCMA | H19F, I22V, Q25S, N31V, L35S, T36V | 432 | 512 |
| 433 BCMA | H19F, Q25G, N31S, L35V, T36N | 433 | 513 |
| 434 BCMA | H19L, Q25H, N31D, L35S | 434 | 514 |
| 435 BCMA | H19F, Q25F, N31S, L35Y, T36S | 435 | 515 |

**[0286]** In some embodiments, also provided herein are BCMA ECD fusion sequences in which any of the above BCMA sequence is linked or fused to a multimerization domain, such as any described herein. Exemplary multimerization domains are described in Section IV.C. In some embodiments, the multimerization domain is an immunoglobulin (e.g. IgG1) Fc region, in which the fusion protein is a BCMA-Fc containing (1) a BCMA sequence containing any of the provided BCMA ECD sequences; and (2) an immunoglobulin Fc region. Thus, among provided embodiments are BCMA-Fc fusion proteins containing (1) a BCMA sequence containing or consisting of any of the above described BCMA ECD polypeptide sequences, such as variant BCMA polypeptide; and (2) an immunoglobulin Fc region. In

some embodiments, the BCMA-Fc fusion is a variant BCMA-Fc fusion containing or consisting of any of the above described variant BCMA polypeptides and an immunoglobulin Fc region.

**[0287]** In some embodiments, provided herein is a variant BCMA-Fc fusion sequence that contains (1) a BCMA ECD sequence that contains the sequence set forth in any one of SEQ ID NOS: 357-435, and (2) an immunoglobulin Fc region. In some embodiments, provided herein is a variant BCMA-Fc fusion sequence that contains (1) a BCMA ECD sequence that consist or consists essentially of the sequence set forth in any one of SEQ ID NOS: 357-435, and (2) an immunoglobulin Fc region.

**[0288]** In some embodiments, provided herein is a variant BCMA-Fc fusion sequence that contains (1) a BCMA ECD sequence that contains the sequence set forth in any one of SEQ ID NOS: 357-435 and (2) an immunoglobulin Fc region. In some embodiments, provided herein is a variant BCMA-Fc fusion sequence that contains (1) a BCMA ECD sequence that consists or consists essentially of the sequence set forth in any one of SEQ ID NOS: 357-435 and (2) an immunoglobulin Fc region.

**[0289]** In provided embodiments of a BCMA-Fc, the immunoglobulin Fc region can be a wild-type Fc of an immunoglobulin, such as an IgG1 Fc. In some cases, the Fc region can be a variant Fc that lacks effector function (also called "effectorless Fc"). Exemplary Fc regions and variants thereof in provided BCMA-Fc fusion proteins are described below in Section IV.C.

**[0290]** In some embodiments, the Fc is murine or human Fc. In some embodiments, the Fc is a mammalian or human IgGl, lgG2, lgG3, or lgG4 Fc regions.

**[0291]** In some embodiments, the Fc is derived from IgG1, such as human IgG1. In some embodiments, the Fc is an IgG1 Fc set forth in SEQ ID NO: 586 having an allotype containing residues Glu (E) and Met (M) at positions 356 and 358 by EU numbering. In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 586 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 586. In other embodiments, the Fc is IgG1 Fc that contains amino acids of the human G1m1 allotype, such as residues containing Asp (D) and Leu (L) at positions 356 and 358, e.g. as set forth in SEQ ID NO:597. Thus, in some cases, an Fc provided herein can contain amino acid substitutions E356D and M358L to reconstitute residues of allotype G1 m1. In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 597 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 597.

**[0292]** In some embodiments, the Fc region has the amino acid sequence set forth in SEQ ID NO:597.

**[0293]** In some embodiments, the variant Fc comprises the sequence set forth in SEQ ID NO: 755. In some embodiments, the variant Fc comprises the sequence set forth in SEQ ID NO:756. In some

embodiments, an Fc region used in a construct provided herein can further lack a C-terminal lysine residue.

**[0294]** In some embodiments, the Fc is derived from IgG2, such as human IgG2. In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 726 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 726.

**[0295]** In some embodiments, the Fc is derived from IgG4, such as human IgG4. In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 727 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 727. In some embodiments, the IgG4 Fc is a stabilized Fc in which the CH3 domain of human IgG4 is substituted with the CH3 domain of human IgG1 and which exhibits inhibited aggregate formation, an antibody in which the CH3 and CH2 domains of human IgG4 are substituted with the CH3 and CH2 domains of human IgG1, respectively, or an antibody in which arginine at position 409 indicated in the EU index proposed by Kabat et al. of human IgG4 is substituted with lysine and which exhibits inhibited aggregate formation (see e.g. U.S. Patent No. 8,911,726. In some embodiments, the Fc is an IgG4 containing the S228P mutation, which has been shown to prevent recombination between a therapeutic antibody and an endogenous IgG4 by Fab-arm exchange (see e.g. Labrijin et al. (2009) Nat. Biotechnol., 27(8): 767-71.) In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 728 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 728.

**[0296]** In some embodiments, the Fc region is a variant Fc region in which a wild-type Fc is modified by one or more amino acid substitutions to reduce effector activity or to render the Fc inert for Fc effector function. Exemplary effectorless or inert mutations include those described herein, including in Section IV.C. In some embodiments, the Fc region of immunomodulatory proteins has an Fc region in which any one or more of amino acids at positions 234, 235, 236, 237, 238, 239, 270, 297, 298, 325, and 329 (indicated by EU numbering) are substituted with different amino acids compared to the native Fc region. Such alterations of Fc region include, for example, alterations such as deglycosylated chains (N297A and N297Q), IgG1-N297G, IgG1-L234A/L235A, IgG1-L234A/L235E/G237A, IgG1-A325A/A330S/P331S, IgG1-C226S/C229S, IgG1-C226S/C229S/E233P/L234V/L235A, IgG1-E233P/L234V/L235A/G236del/ S267K, IgG1-L234F/L235E/P331S, IgG1-S267E/L328F, IgG2-V234A/G237A, IgG2-H268Q/V309L/A330S/A331S, IgG4-L235A/G237A/E318A, and IgG4-L236E described in Current Opinion in Biotechnology (2009) 20 (6), 685-691; alterations such as G236R/L328R, L235G/G236R, N325A/L328R, and N325LL328R described in WO 2008/092117; amino

acid insertions at positions 233, 234, 235, and 237 (indicated by EU numbering); and alterations at the sites described in WO 2000/042072.

**[0297]** In some embodiments, the variant Fc region comprises the one or more amino acid modifications (e.g amino acid substitutions) is derived from a wild-type IgG1, such as a wild-type human IgG1. In some embodiments, the wild-type IgG1 Fc can be the Fc set forth in SEQ ID NO: 586 having an allotype containing residues Glu (E) and Met (M) at positions 356 and 358 by EU numbering. In some embodiments, the variant Fc region is derived from the amino acid sequence set forth in SEQ ID NO: 586. In other embodiments, the wild-type IgG1 Fc contains amino acids of the human G1m1 allotype, such as residues containing Asp (D) and Leu (L) at positions 356 and 358, e.g. as set forth in SEQ ID NO:597. Thus, in some cases, the variant Fc is derived from the amino acid sequence set forth in SEQ ID NO:597.

**[0298]** In some embodiments, the Fc region lacks the C-terminal lysine corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 586 or 597 (corresponding to K447del by EU numbering).

**[0299]** In some embodiments, the variant Fc region comprises a C5S amino acid modification of the wild-type or unmodified Fc region by numbering of SEQ ID NO: 586 (corresponding to C220S by EU numbering).

**[0300]** In some embodiments, the Fc region is a variant Fc that contains at least one amino acid substitution that is N82G by numbering of SEQ ID NO: 586 (corresponding to N297G by EU numbering). In some embodiments, the Fc further contains at least one amino acid substitution that is R77C or V87C by numbering of SEQ ID NO: 586 (corresponding to R292C or V302C by EU numbering). In some embodiments, the variant Fc region further comprises a C5S amino acid modification by numbering of SEQ ID NO: 586 (corresponding to C220S by EU numbering). For example, in some embodiments, the variant Fc region comprises the following amino acid modifications: N297G and one or more of the following amino acid modifications C220S, R292C or V302C by EU numbering (corresponding to N82G and one or more of the following amino acid modifications C5S, R77C or V87C with reference to SEQ ID NO:586), e.g., the Fc region comprises the sequence set forth in SEQ ID NO:598.

**[0301]** In some embodiments, the variant Fc contains the amino acid substitutions L234A/L235E/G237A, by EU numbering. In some embodiments, the variant Fc contains the amino acid substitutions A330S/P331S, by EU numbering. In some embodiments, the variant Fc contains the amino acid substitutions L234A/L235E/G237A/ A330S/P331S (Gross et al. (2001) Immunity 15:289). In some embodiments, the variant Fc comprises the sequence set forth in SEQ ID NO: 757. In some embodiments, the variant Fc comprises the sequence set forth in SEQ ID NO:758. In some

embodiments, an Fc region used in a construct provided herein can further lack a C-terminal lysine residue.

**[0302]** In some embodiments, the Fc region is a variant Fc that includes mutations L234A, L235E and G237A by EU numbering. In some embodiments, a wild-type Fc is further modified by the removal of one or more cysteine residue, such as by replacement of the cysteine residues to a serine residue at position 220 (C220S) by EU numbering. Exemplary inert Fc regions having reduced effector function are set forth in SEQ ID NO: 599 and SEQ ID NO:591, which are based on allotypes set forth in SEQ ID NO:586 or SEQ ID NO: 597, respectively. In some embodiments, an Fc region can further lack a C-terminal lysine residue. In some embodiments, the variant Fc region comprises one or more of the amino acid modifications C220S, L234A, L235E or G237A, e.g. the Fc region comprises the sequence set forth in SEQ ID NO:589, 591, 599 or 724. In some embodiments, the variant Fc comprises has the sequence set forth in SEQ ID NO: 589. In some embodiments, the variant Fc comprises has the sequence set forth in SEQ ID NO: 591. In some embodiments, the variant Fc comprises has the sequence set forth in SEQ ID NO: 599. In some embodiments, the variant Fc comprises has the sequence set forth in SEQ ID NO: 724.

**[0303]** In some embodiments, the Fc region is a variant Fc that has the sequence set forth in SEQ ID NO:589.

**[0304]** In some embodiments, the Fc region is a variant Fc region that comprises one or more of the amino acid modifications C220S, L235P, L234V, L235A, G236del or S267K, e.g. the Fc region comprises the sequence set forth in SEQ ID NO:722. In some embodiments, the Fc region lacks the C-terminal lysine corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 586 (corresponding to K447del by EU numbering).

**[0305]** In some embodiments, the Fc region is a variant Fc region that comprises one or more of the amino acid modifications C220S, R292C, N297G, V302C. In some embodiments, the Fc region lacks the C-terminal lysine corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 586 (corresponding to K447del by EU numbering). An exemplary variant Fc region is set forth in SEQ ID NO: 723.

**[0306]** In some embodiments, the variant Fc region comprises one or more of the amino acid modifications C220S/E233P/L234V/L235A/G236del/S267K. In some embodiments, the Fc region lacks the C-terminal lysine corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 586 (corresponding to K447del by EU numbering). An exemplary variant Fc region is set forth in SEQ ID NO: 725.

**[0307]** In some embodiments, the Fc region is a variant Fc region containing any combination of the Fc mutations in Table 4. In some embodiments, the Fc region is a variant Fc region having the sequence set forth in any one of the SEQ ID NOs in Table 4.

**[0308]** For example, a variant Fc region may be an effectorless Fc that exhibits reduced effector activity compared to a wild-type IgG1 set forth in SEQ ID NO:586 or SEQ ID NO:597. In some embodiments, the variant Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS:591, 598, 599, 722, 589, 723, 724, or 725 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 591, 598, 599722, 589, 723, 724, or 725. In some embodiments, the variant Fc has the sequence set forth in SEQ ID NO: 589.

**[0309]** In some embodiments, the BCMA polypeptide, such as the variant BCMA polypeptide, is directly linked to the Fc sequence. In some embodiments, the BCMA polypeptide, such as the variant BCMA polypeptide, is indirectly linked to the Fc sequence, such as via a linker. In some embodiments, one or more "peptide linkers" link the BCMA polypeptide (e.g. variant BCMA polypeptide) and the Fc region. In some embodiments, a peptide linker can be a single amino acid residue or greater in length. In some embodiments, the peptide linker has at least one amino acid residue but is no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues in length. Exemplary linkers are set forth in subsection "Linker."

**[0310]** In some embodiments, the linker is (in one-letter amino acid code): GGGGS ("4GS"; SEQ ID NO: 593) or multimers of the 4GS linker, such as repeats of 2, 3, 4, or 5 4GS linkers. In some embodiments, the peptide linker is the peptide linker is $(GGGGS)_2$ (SEQ ID NO: 594), $(GGGGS)_3$ (SEQ ID NO: 595), $(GGGGS)_4$ (SEQ ID NO: 600) or $(GGGGS)_5$ (SEQ ID NO: 671). In some embodiments, the linker also can include a series of alanine residues alone or in addition to another peptide linker (such as a 4GS linker or multimer thereof). In some embodiments, the linker (in one-letter amino acid code) is GSGGGGS (SEQ ID NO: 590) or GGGGSSA (SEQ ID NO: 596). In some examples, the linker is a 2xGGGGS followed by three alanines (GGGGSGGGGSAAA; SEQ ID NO:721).

**[0311]** In some embodiments, there is a provided a BCMA-Fc fusion protein that is a dimer formed by two identical BCMA Fc polypeptides (e.g. variant BCMA polypeptide) as described linked to an Fc domain. In some embodiments, identical species of any of the provided BCMA-Fc fusion polypeptides, e.g. variant BCMA-Fc fusion, will be dimerized to create a homodimer. In some embodiments, the dimer is a homodimer in which the two BCMA Fc polypeptides, e.g. variant BCMA Fc polypeptides, are the same. For generating a homodimeric Fc molecule, the Fc region is one that is capable of forming a homodimer with a matched Fc region by co-expression of the individual Fc regions in a cell.

**[0312]** Also provided are nucleic acid molecules encoding the BCMA-Fc fusion proteins, e.g. variant BCMA-Fc fusion protein. In some embodiments, for production of an Fc fusion protein, a nucleic acid molecule encoding a BCMA-Fc fusion protein, e.g. variant BCMA-Fc fusion protein is inserted into an appropriate expression vector. The resulting BCMA-Fc fusion protein, e.g. variant BCMA-Fc fusion protein can be expressed in host cells transformed with the expression where assembly between Fc

domains occurs by interchain disulfide bonds formed between the Fc moieties to yield dimeric, such as divalent, BCMA-Fc fusion proteins. The resulting Fc fusion proteins can be easily purified by affinity chromatography over Protein A or Protein G columns.

**[0313]** In embodiments, when produced and expressed from a cells, the provided immunomodulatory protein, such as a BCMA-Fc, is a homodimer containing two identical polypeptide chains. **FIG. 15** depicts the structure of an exemplary BCMA-Fc fusion protein provided herein.

## III. MULTI-DOMAIN IMMUNOMODULATORY PROTEINS

**[0314]** Provided herein are multi-domain immunomodulatory proteins that contains (1) one or more B cell inhibitory molecule (BIM) that bind to a ligand of a B cell stimulatory receptor and (2) one or more T cell inhibitory molecule (BIM) that binds to a T cell stimulatory receptor or a ligand of a T cell stimulatory receptor. Among provided multi-domain immunomodulatory proteins are those in which the BIM antagonizes, such as reduces or inhibits, the activity of a B cell stimulatory receptor, and in which the TIM antagonizes, such as reduces or inhibits, the activity of a T cell stimulatory receptor. Thus, the provided immunomodulatory proteins combine B and T cell inhibitors into a single molecule. In some embodiments, the BIM and TIM are linked directly or indirectly. The provided immunomodulatory protein can be a fusion protein in which the multidomain BIM and TIM components are further linked to another moiety, such as a multimerization domain or half-life extending molecule. In particular embodiments, the multidomain immunomodulatiory protein is a BIM/TIM Fc fusion protein. The provided molecules can be used to modulate B and T cell pathways to thereby treat autoimmune diseases, particularly automimmune diseases in which etiology is associated with B and T cell responses.

**[0315]** In some embodiments, the B cell stimulatory receptor is a receptor expressed on B cells that stimulates B cell responses, such as B cell maturation and differentiation. The B cell stimulatory receptor may be BAFF-R, BCMA and/or TACI. In particular embodiments, the provided multidomain immunomodulatory proteins antagonize the activity of one or more of BAFF-R, BCMA or TACI. In some embodiments, the BIM binds to a ligand of BAFF-R, BCMA or TACI. The ligand may be BAFF or APRIL, which are homotrimeric molecules common to members of the TNF superfamily. BAFF and APRIL are both mainly expressed by myeloid cells, and have been reported to act as costimulatory B cell factors. BAFF and APRIL share two receptors TACI and BCMA; BAFF is also able to bind and stimulate BAFF-R. In some cases, the ligand may be a heterotrimer of BAFF and APRIL. For example, heterotrimeric complexes of APRIL and BAFF are found in serum, particularly in subjects with autoimmune disease such as those with systemic immune-based rheumatic diseases.

**[0316]** In some embodiments, the T cell stimulatory receptors comprises an immunoreceptor tyrosine-based activation motif (ITAM) or interacts with an adaptor protein involved in signal transduction pathways in a T cell to transduce activation signals. The T cell stimulatory receptor may be

a costimulatory receptor expressed on T cells, such as CD28 or ICOS. In particular embodiments, the provided multidomain immunomodulatory proteins antagonize the activity of the T cell stimulatory receptor, such as a T cell costimulatory receptor, e.g. CD28 or ICOS. The TIM may bind to the costimulatory receptor or to a ligand of the costimulatory receptor. In some embodiments, the provided multi-domain immunomodulatory proteins can be generated so that the TIM binds the T cell stimulatory receptor directly. For example, the TIM may be a CD28 or ICOS binding molecule. In other embodiments, the provided multi-domain immunomodulatory proteins can be generated so that the TIM binds a ligand of the T cell stimulatory receptor, and thereby indirectly antagonizes or inhibits the T cell stimulatory receptor. For example, the TIM binds to CD80 or CD86, which are ligands of CD28.

**[0317]** In some embodiments, the one or more TIM and/or BIM independently include an antibody or an antigen-binding antibody fragment. In some aspects, the TIM and/or BIM can be a human antibody and/or an antibody that binds a human protein.

**[0318]** In some embodiments, at least one of the TIM or BIM is not an antibody or antigen-binding fragment. In some embodiments, at least one of the TIM or BIM is or contains an extracellular domain of a cell surface molecule expressed on immune cells. For example, certain members of the non-antibody immunoglobulin superfamily (IgSF) are expressed on T cells or regulate activity of T cells. These include, for example, certain T cell costimulatory molecules or ligands thereof. In some cases, the TIM includes an (IgSF) domain (IgD) of an IgSF member (e.g. wild-type IgD), or a variant IgD (hereinafter called "vIgD") in which is contained one or more amino acid modifications (e.g. substitutions) in an IgD. Likewise, certain members of the TNF receptor superfamily are expressed on B cells or regulate activity of B cells. These include, for example, certain B cell stimulatory receptors, such as TACI or BCMA. In some cases, the BIM include a TNF receptor domain (TD) of a TNFR superfamily member (e.g. wild-type TD), or a variant TD (hereinafter called "vTD") in which is contained one more amino acid modifications (e.g. substitutions) in an TD.

**[0319]** In some embodiments, the BIM can bind to a ligand of a B cell stimulatory receptor with at least a certain binding activity, such as binding affinity, as measured by any of a number of known methods. In some embodiments, the TIM can bind to a T cell stimulatory receptor or a ligand of a T cell stimulatory receptor with at least a certain binding activity, such as binding affinity, as measured by any of a number of known methods. In some embodiments, the affinity is represented by an equilibrium dissociation constant ($K_D$) or is represented by $EC_{50}$. A variety of assays are known for assessing binding activity, including binding affinity, and/or determining whether a binding molecule (*e.g.*, a TIM or BIM) specifically binds to a particular binding partner. In some embodiments, a BIAcore® instrument can be used to determine the binding kinetics and constants of a complex between two proteins using surface plasmon resonance (SPR) analysis (*see*, *e.g.*, Scatchard *et al.*, *Ann. N.Y. Acad. Sci. 51*:660, 1949; Wilson, *Science 295*:2103, 2002; Wolff *et al.*, *Cancer Res. 53*:2560, 1993; and U.S. Patent Nos. 5,283,173,

5,468,614, or the equivalent). In other embodiments, Bio-Layer Interferometry (BLI) using a ForteBio Octet system may be used, such as with streptavidin coated sensor and biotinylated recombinant protein domain. Other suitable assays for measuring the binding of one protein to another include, for example, immunoassays such as enzyme linked immunosorbent assays (ELISA) and radioimmunoassays (RIA), or determination of binding by monitoring the change in the spectroscopic or optical properties of the proteins through fluorescence, UV absorption, circular dichroism, or nuclear magnetic resonance (NMR). Other exemplary assays include, but are not limited to, Western blot, ELISA, analytical ultracentrifugation, spectroscopy, flow cytometry, sequencing and other methods for detection of expressed nucleic acids or binding of proteins.

**[0320]** In some embodiments, the BIM and TIM independently exhibit a binding affinity for a binding partner with a $K_D$ (*i.e.*, an equilibrium dissociation constant of a particular binding interaction with units of M; equal to the ratio of the off-rate [$k_{off}$ or $k_d$] to the on-rate [$k_{on}$ or $k_a$] for this association reaction, assuming bimolecular interaction) of equal to or less than $10^{-5}$ M. For example, the equilibrium dissociation constant $K_D$ ranges from $10^{-6}$ M to $10^{-12}$ M, such as $10^{-7}$ M to $10^{-11}$ M, $10^{-8}$ M to $10^{-10}$ M, or $10^{-9}$ M to $10^{-10}$ M. The on-rate (association rate constant; $k_{on}$ or $k_a$; units of 1/Ms) and the off-rate (dissociation rate constant; $k_{off}$ or $k_d$; units of 1/s) can be determined using any of the assay methods known in the art, for example, surface plasmon resonance (SPR).

**[0321]** In some embodiments, the BIM exhibits a binding affinity for a ligand of a B cell stimulatory receptor that is from or from about 0.001 nM to 1000 nM, such as from or from about 0.01 nM to about 500 nM, from or from about 0.01 nM to about 400 nM, from or from about 0.01 nM to about 100 nM, from or from about 0.01 nM to about 50 nM, from or from about 0.01 nM to about 10 nM, from or from about 0.01 nM to about 1 nM, from or from about 0.01 nM to about 0.1 nM, is from or from about 0.1 nM to about 500 nM, from or from about 0.1 nM to about 400 nM, from or from about 0.1 nM to about 100 nM, from or from about 0.1 nM to about 50 nM, from or from about 0.1 nM to about 10 nM, from or from about 0.1 nM to about 1 nM, from or from about 0.5 nM to about 200 nM, from or from about 1 nM to about 500 nM, from or from about 1 nM to about 100 nM, from or from about 1 nM to about 50 nM, from or from about 1 nM to about 10 nM, from or from about 2 nM to about 50 nM, from or from about 10 nM to about 500 nM, from or from about 10 nM to about 100 nM, from or from about 10 nM to about 50 nM, from or from about 50 nM to about 500 nM, from or from about 50 nM to about 100 nM or from or from about 100 nM to about 500 nM. In certain embodiments, the binding affinity of the BIM for the inhibitory receptor is at or less than or about 400 nM, 300 nM, 200 nM, 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM or less.

**[0322]** In some embodiments, the TIM exhibits a binding affinity for a T cell stimulatory receptor or a ligand of a T cell stimulatory receptor that is from or from about 0.001 nM to about 1000 nM, such as

from or from about 0.01 nM to about 500 nM, from or from about 0.01 nM to about 400 nM, from or from about 0.01 nM to about 100 nM, from or from about 0.01 nM to about 50 nM, from or from about 0.01 nM to about 10 nM, from or from about 0.01 nM to about 1 nM, from or from about 0.01 nM to about 0.1 nM, is from or from about 0.1 nM to about 500 nM, from or from about 0.1 nM to about 400 nM, from or from about 0.1 nM to about 100 nM, from or from about 0.1 nM to about 50 nM, from or from about 0.1 nM to about 10 nM, from or from about 0.1 nM to about 1 nM, from or from about 0.5 nM to about 200 nM, from or from about 1 nM to about 500 nM, from or from about 1 nM to about 100 nM, from or from about 1 nM to about 50 nM, from or from about 1 nM to about 10 nM, from or from about 2 nM to about 50 nM, from or from about 10 nM to about 500 nM, from or from about 10 nM to about 100 nM, from or from about 10 nM to about 50 nM, from or from about 50 nM to about 500 nM, from or from about 50 nM to about 100 nM or from or from about 100 nM to about 500 nM. In certain embodiments, the binding affinity of the TIM for the stimulatory receptor or a ligand of the T cell stimulatory receptor is at or less than or about 400 nM, 300 nM, 200 nM, 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM or less.

**[0323]** In some embodiments, the TIM, or the multidomain immunomodulatory protein containing the TIM, is not an agonist of the T cell stimulatory (e.g. costimulatory) receptor. In some embodiments, the TIM, or the multidomain immunodulatory protein containing the TIM, binds to a T cell costimulatory receptor, e.g. CD28 or ICOS, but exhibits a relatively low affinity for the T cell costimulatory receptor. In some embodiments, the TIM has a binding affinity for the T cell costimulatory receptor of greater than $1 \times 10^{-9}$ M, such as between at or about $1 \times 10^{-7}$ M and at or about $1 \times 10^{-9}$ M. In some embodiments, the TIM has a binding affinity for the T cell costimulatory receptor of at or about $1 \times 10^{-7}$ M, at or about $2.5 \times 10^{-7}$ M, at or about $5 \times 10^{-7}$ M, at or about $7.5 \times 10^{-7}$ M, at or about $1 \times 10^{-8}$ M, at or about $2.5 \times 10^{-8}$ M, at or about $5 \times 10^{-8}$ M, at or about $7.5 \times 10^{-8}$ M, or at or about $1 \times 10^{-9}$ M, or any value between any of the foregoing.

**[0324]** In some embodiments, the TIM of the provided multidomain immunomodulatory proteins does not directly bind to a T cell costimulatory receptor. In some embodiments, the TIM of the provided multidomain immunomodulatory proteins binds to a ligand of the T cell costimulatory receptor.

**[0325]** In some embodiments, the provided multi-domain immunomodulatory proteins can include the BIM and TIM in various configurations or formats, including formats with one or more further moieties. In some embodiments, the provided immunomodulatory proteins include polypeptides in which the one or more BIM is N-terminal to the TIM. In some embodiments, the one or more BIM is C-terminal to the TIM. The one or more BIM and the one or more TIM can be linked directly or indirectly, via a linker. In some embodiments, the immunomodulatory proteins can be formatted as multimeric molecules via fusion with a multimerization domain, such as an Fc protein. In some embodiments, the

multi-domain immunomodulatory proteins can be formatted as multimeric molecules, e.g., dimeric, trimer, tetrameric, or pentameric molecules. In some embodiments, the immunomodulatory proteins are formatted as a monomeric molecules containing single polypeptide fusions of the one or more BIM and the one or more TIM. **FIG. 16** depicts exemplary formats and configurations, all of which may be encompassed by a provided multi-domain immunomodulatory protein.

**[0326]** In the subsections below, exemplary BIM and TIM components of the provided multi-domain immunomodulatory protein are described, as are exemplary formats for such immunomodulatory proteins.

### A. B CELL INHIBITORY MOLECULE (BIM)

**[0327]** In some embodiments, the provided immunomodulatory protein contains a BIM that binds to one or more ligands of a B cell stimulatory receptor. In some embodiments, the B cell stimulatory receptor is a member of the TNFRSF. In some embodiments, the one or more B cell stimulatory receptor is TACI and BCMA. In some embodiments, the ligand of the B cell stimulatory receptor is BAFF or APRIL. In some embodiments the BIM binds to BAFF, APRIL and/or a BAFF/APRIL heterotrimer. In some embodiments the BIM is able to binds to BAFF, APRIL and a BAFF/APRIL heterotrimer.

**[0328]** In some embodiments, the BIM is an antibody or antigen-binding fragment that binds to the ligand of a B cell stimulatory receptor. In some embodiments, the BIM is an antibody or antigen-binding fragment that binds BAFF and/or APRIL, such as a human BAFF and/or human APRIL.

**[0329]** In some embodiments, the BIM is or contains a binding partner of the ligand of the B cell stimulatory receptor. In some embodiments, the multi-domain immunomodulatory protein provided herein are soluble proteins and/or do not contain a portion that includes a transmembrane domain. Those of skill will appreciate that cell surface proteins, including proteins of the TNFRSF such as B cell stimulatory receptors, e.g. BCMA and TACI, typically have an intracellular domain, a transmembrane domain, and extracellular domain (ECD), and that a soluble form of such proteins can be made using the extracellular domain or an immunologically active subsequence thereof. Thus, in some embodiments, the BIM lacks a transmembrane domain or a portion of the transmembrane domain of the B cell stimulatory receptor, e.g. BCMA or TACI. In some embodiments, the BIM lacks the intracellular (cytoplasmic) domain or a portion of the intracellular domain of the B cell stimulatory receptor, e.g. BCMA or TACI. In some embodiments, the BIM only contains the ECD domain or a portion thereof containing a TD, such as a CRD, or specific binding fragments thereof.

**[0330]** For example, in some aspects, the BIM is or contains an ECD of a B cell stimulatory receptor, or a specific binding portion or fragment thereof containing at least one TD (e.g. at least one CRD), that binds to a ligand of the B cell stimulatory receptor. For example, the BIM can contain an ECD of TACI or BCMA, or a specific binding portion or fragment of TACI or BCMA containing at least

one TD (e.g. at least one CRD), that binds to APRIL, BAFF and/or an APRIL/BAFF heterotrimer. In some embodiments, the BIM consists or consists essentially of an ECD of a B cell stimulatory receptor, or a specific binding portion or fragment thereof containing at least one TD (e.g. at least one CRD), such as consists or consists essentially of the ECD of TACI or BCMA or a specific binding portion or fragment of the ECD of TACI or BCMA that contains at least one TD (e.g. at least one CRD). In some embodiments, the BIM is less than the full length sequence of the ECD of the B cell stimulatory receptor. In some embodiments, the BIM is or only contains one CRD or a specific binding fragment of the CRD. In some embodiments, the BIM consists or consists essentially of a CRD of a B cell stimulatory receptor, such as consists or consists essentially of only one CRD of TACI or BCMA. In some embodiments, the sequence of the BIM containing an ECD or binding portion or fragment thereof containing a TD (e.g. at least one CRD) is a mammalian sequence that includes, but is not limited to, human, mouse, cynomolgus monkey, or rat. In some embodiments, the BIM sequence is human and/or binds a human protein.

**[0331]** In some aspects, the BIM is or includes a vTD that is an affinity-modified domain that exhibits increased binding activity, such as increased binding affinity, for the ligand of the B cell stimulatory receptor compared to the binding activity of the unmodified or wild-type TD for the same molecule. In some embodiment, the BIM contains a vTD with one or more amino acid substitutions compared to a TD of a TNFRSF member, e.g. BCMA or TACI, in which, the one or more amino acid substitutions confer or result in increased binding affinity to a cognate ligand of the B cell stimulatory receptor.

**[0332]** In some embodiments, the BIM is or contains a vTD that contains one or more amino acids modifications, such as one or more substitutions (alternatively, "mutations" or "replacements"), deletions or additions, relative to a wild-type or unmodified TD of a binding partner of a ligand of the B cell stimulatory receptor. In some aspects, the vTD contains up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid modifications, such as amino acid substitutions, deletions or additions in an TD domain of an TNFRSF binding partner of a B cell stimulatory receptor. The modifications (e.g., substitutions) can be in a CRD. In some embodiments, the vTD has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modifications (e.g., substitutions) in the CRD or specific binding fragment thereof. In some embodiments, the vTD has at least about 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the wild-type or unmodified TD or specific binding fragment thereof.

**[0333]** Non-limiting examples of a BIM in the provided multidomain immunomodulatory proteins are described in the following subsections. Any of the described BIMs herein can be combined with a TIM as described in Section III.B.

### *I. TACI*

**[0334]** In some embodiments, the BIM is or contains a wild-type TACI ECD or a specific binding portion or fragment thereof containing at least one TD (e.g. at least one CRD) that binds to APRIL, BAFF and/or an APRIL/BAFF heterotrimer. In some embodiments, the BIM is or contains a variant TACI ECD or a specific binding portion or fragment thereof containing at least one TD (e.g. at least one CRD) that binds to APRIL, BAFF and/or an APRIL/BAFF heterotrimer. In some embodiments, the BIM is a TACI polypeptide or variant thereof with any of the sequences set forth herein.

**[0335]** TACI is a tumor necrosis factor receptor family member characterized by having an extracellular domain (ECD) containing cysteine-rich pseudo-repeat domains (CRDs). TACI is a membrane bound receptor, which has an extracellular domain containing two cysteine-rich pseudo-repeats (CRD1 and CRD2), a transmembrane domain and a cytoplasmic domain that interacts with CAML (calcium-modulator and cyclophilin ligand), an integral membrane protein located at intracellular vesicles which is a co-inducer of NF-AT activation when overexpressed in Jurkat cells. TACI is associated with B cells and a subset of T cells. The TACI receptor binds two members of the tumor necrosis factor (TNF) ligand family. One ligand is designated BAFF (B cell Activating Factor of the TNF Family), and also is variously designated as ZTNF4, "neutrokine-α," "BLyS," "TALL-1," and "THANK" (Yu et al., international publication No. WO98/18921 (1998), Moore et al., Science 285:269 (1999); Mukhopadhyay et al., J. Biol. Chem. 274:15978 (1999); Schneider et al., J. Exp. Med. 189:1747 (1999); Shu et al., J. Leukoc. Biol. 65:680 (1999)). The other ligand has been designated as APRIL, and also is variously designated as "ZTNF2" and "TNRF death ligand-1" (Hahne et al., J. Exp. Med. 188:1185 (1998); Kelly et al., Cancer Res. 60:1021 (2000)). Both ligands are also bound by the B-cell maturation receptor (BCMA) (Gross et al., Nature 404:995 (2000)). Binding of TACI receptor to its ligands BAFF or APRIL stimulates B cell responses, including T cell-independent B cell antibody responses, isotype switching, and B cell homeostasis.

**[0336]** The amino acid sequence of full-length TACI is set forth in SEQ ID NO:666. The protein is a type III membrane protein and lacks a signal peptide; following expression in eukaryotic cells the N-terminal methionine is removed. In some embodiments, a mature TACI protein does not contain the N-terminal methionine as set forth in SEQ ID NO:666. The extracellular domain of TACI (amino acid residues 1-166 of SEQ ID NO:666; ECD set forth in SEQ ID NO:709) contains two cysteine rich domain (CRDs, hereinafter also called a tumor necrosis family receptor domain or TD), each of which exhibit affinity for binding to BAFF and APRIL. The first cysteine rich domain (CRD1) contains amino acid residues 34-66 of the sequence set forth in SEQ ID NO:709. The second cysteine rich domain (CRD2) corresponds to amino acids 71-104 of the sequence set forth in SEQ ID NO:709. TACI also contains a stalk region of about 60 amino acids following the second cysteine repeat in the extracellular domain, corresponding to amino acid residues 105 -165 of the sequence set forth in SEQ ID NO:709.

**[0337]** In some embodiments, the BIM is a variant TACI polypeptide that contains one or more amino acid modifications, such as one or more substitutions (alternatively, "mutations" or "replacements"), deletions or additions in the extracellular domain of a reference TACI polypeptide, such as a wild-type or unmodified TACI polypeptide containing a CRD(s) (hereinafter also called TDs). Thus, a provided BIM that is a variant TACI polypeptide is or comprises a variant TD ("vTD") in which the one or more amino acid modifications (e.g. substitutions) is in a CRD. In some embodiments, the one or more amino acids modifications, such as one or more substitutions (alternatively, "mutations" or "replacements"), deletions or addition, is in the CRD1 region. In some embodiments, the one or more amino acids modifications, such as one or more substitutions (alternatively, "mutations" or "replacements"), deletions or addition, is in the CRD2 region. In some embodiments, the one or more amino acids modifications, such as one or more substitutions (alternatively, "mutations" or "replacements"), deletions or addition, is in amino acids within both the CRD1 and CRD2 regions.

**[0338]** In some embodiments, the reference (e.g. unmodified) TACI sequence is a wild-type TACI sequence or is a portion thereof that contains one or both CRDs. In some embodiments, the reference (e.g., unmodified) TACI is or comprises the extracellular domain (ECD) of TACI or a portion thereof containing one or both CRD domains. In some embodiments, the extracellular domain of a reference (e.g., unmodified) TACI polypeptide comprises a CRD1 and CRD2. However, the variant TACI polypeptide need not comprise both the CRD1 and the CRD2. In some embodiments, the variant TACI polypeptide comprises or consists essentially of the CRD1 or a specific binding fragment thereof. In some embodiments, the variant TACI polypeptide comprises or consists essentially of the CRD2 or specific binding fragments thereof. In some embodiments, the variant TACI is a soluble polypeptide and lacks a transmembrane domain. In some embodiments, the variant TACI polypeptide further comprises a transmembrane domain and, in some cases, also a cytoplasmic domain.

**[0339]** In some embodiments, the reference (e.g., unmodified) TACI sequence is a mammalian TACI sequence. In some embodiments, the reference (e.g., unmodified) TACI sequence can be a mammalian TACI that includes, but is not limited to, human, mouse, cynomolgus monkey, or rat. In some embodiments, the reference (e.g., unmodified) TACI sequence is human. The extracellular domain of an exemplary human TACI sequence is set forth in SEQ ID NO:709.

**[0340]** In some embodiments, the reference (e.g., unmodified) TACI sequence has (i) the sequence of amino acids set forth in SEQ ID NO:709 or a sequence thereof that lacks the N-terminal methionine, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:709 and that binds to APRIL, BAFF or an APRIL/BAFF heterotrimer, or (iii) is a fragment or portion of (i) or (ii) containing a CRD1 and/or CRD2, in which the portion binds to APRIL, BAFF or an APRIL/BAFF heterotrimer . In

some embodiments, the reference (e.g., unmodified) TACI sequence lacks the N-terminal methionine as set forth in SEQ ID NO: 709.

**TACI Extracellular Domain (ECD): SEQ ID NO:709**

MSGLGRSRRGGRSRVDQEERFPQGLWTGVAMRSCPEEQYWDPLLGTCMSCKTICNHQSQ
RTCAAFCRSLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRSPVNLPPELRR
QRSGEVENNSDNSGRYQGLEHRGSEASPALPGLKLSADQVALVYST

**[0341]** In some embodiments, the reference (e.g. unmodified) TACI sequence is an extracellular domain sequence of TACI that is a portion of the ECD that contains an N-terminal deletion relative to the sequence of amino acids set forth in SEQ ID NO:709. In some embodiments, the N-terminal deletion is deletion of N-terminal amino acid residues 1-28 corresponding to residues set forth in SEQ ID NO:709. In some embodiments, the N-terminal deletion is deletion of N-terminal amino acid residues 1-29 corresponding to residues set forth in SEQ ID NO:709. In some embodiments, the N-terminal deletion is deletion of N-terminal amino acid residues 1-30 corresponding to residues set forth in SEQ ID NO:709. In some embodiments, the N-terminal deletion is deletion of N-terminal amino acid residues 1-31 corresponding to residues set forth in SEQ ID NO:709. In some embodiments, the N-terminal deletion is deletion of N-terminal amino acid residues 1-32 corresponding to residues set forth in SEQ ID NO:709. In some embodiments, the N-terminal deletion is deletion of N-terminal amino acid residues 1-33 corresponding to residues set forth in SEQ ID NO:709.

**[0342]** In some of any of the provided embodiments, the reference (e.g. unmodified) TACI sequence is an ECD portion that contains deletion of one or more residues of the stalk portion of the TACI extracellular domain. In some embodiments, the reference (e.g. unmodified) TACI sequence is an ECD portion that lacks one or more contiguous C-terminal amino acid residues beginning at residue 105 and up to or including amino acid residue 166 corresponding to residues of the ECD sequence set forth in SEQ ID NO:709. In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61 or 62 of the ECD sequence is deleted.

**[0343]** In some embodiments, the reference (e.g. unmodified) TACI sequence contains an ECD portion having a contiguous sequence of amino acids that includes the CRD1 and/or CRD2 (e.g. CRD1 and CRD2 or CRD2 only) and only a segment or portion of the stalk sequence. Suitable stalk segments include one or more amino acids of amino acid residues 105 to 154 of SEQ ID NO:709. For example, the stalk segment can consist of the following with reference to SEQ ID NO:709: amino acid residue 105, amino acid residues 105 to 106, amino acid residues 105 to 107, amino acid residues 105 to 108, amino acid residues 105 to 109, amino acid residues 105 to 110, amino acid residues 105 to 111, amino acid residues 105 to 112, amino acid residues 105 to 113, amino acid residues 105 to 114, amino acid residues 105 to 115, amino acid residues 105 to 116, amino acid residues 105 to 117, amino acid residues 105 to

118, amino acid residues 105 to 119, amino acid residues 105 to 120, amino acid residues 105 to 121, amino acid residues 105 to 122, amino acid residues 105 to 123, amino acid residues 105 to 124, amino acid residues 105 to 125, amino acid residues 105 to 126, amino acid residues 105 to 127, amino acid residues 105 to 128, amino acid residues 105 to 129, amino acid residues 105 to 130, amino acid residues 105 to 131, amino acid residues 105 to 132, amino acid residues 105 to 133, amino acid residues 105 to 134, amino acid residues 105 to 135, amino acid residues 105 to 136, amino acid residues 105 to 137, amino acid residues 105 to 138, amino acid residues 105 to 139, amino acid residues 105 to 140, amino acid residues 105 to 141, amino acid residues 105 to 142, amino acid residues 105 to 143, amino acid residues 105 to 144, amino acid residues 105 to 145, amino acid residues 105 to 146, amino acid residues 105 to 147, amino acid residues 105 to 148, amino acid residues 105 to 149, amino acid residues 105 to 150, amino acid residues 105 to 151, amino acid residues 105 to 152, amino acid residues 105 to 153, and amino acid residues 105 to 154.

**[0344]** In some embodiments, the reference (e.g. unmodified) TACI sequence lacks or is mutated in one or more potential furin cleavage sites. In some cases, the reference (e.g. unmodified) TACI sequence is an ECD or portion that in which the arginine residue at position 119 is mutated, e.g. R119G. In some cases, the reference (e.g. unmodified) TACI sequence is an ECD or portion that in which the glutamine residue at position 121 is mutated, e.g. Q121P. In some cases, the reference (e.g. unmodified) TACI sequence is an ECD or portion that in which the arginine residue at position 122 is mutated, e.g. R122Q.

**[0345]** In some embodiments, the reference TACI sequence is a TACI ECD sequence as set forth in international PCT publication No. WO2000/067034, WO2002/094852 or WO2008/154814.

**[0346]** In some embodiments, the reference TACI sequence is a TACI ECD sequence that has or consists of the sequence set forth in SEQ ID NO:719.

**TACI ECD (CRD1/CRD2): SEQ ID NO:719**

SRVDQEER FPQGLWTGVA MRSCPEEQYW DPLLGTCMSCKTICNHQSQR TCAAFCRSLS CRKEQGKFYD HLLRDCISCA SICGQHPKQCAYFCENKLRS PVNLPPEL

**[0347]** In some embodiments, the reference TACI sequence is a TACI ECD sequence that has or consists of the sequence set forth in SEQ ID NO:718.

**TACI ECD (CRD1/CRD2): SEQ ID NO:718**

AMRSCPEEQYWDPLLGTCMSCKTICNHQSQRTCAAFCRSLSCRKEQGKFYDHLLRDCISC ASICGQHPKQCAYFCENKLRS

**[0348]** In some embodiments, the reference TACI sequence is a TACI ECD sequence that has or consists of the sequence set forth in SEQ ID NO:516 (encoded by the sequence of nucleotides set forth in SEQ ID NO:551).

**TACI ECD (CRD1/CRD2): SEQ ID NO:516**

VAMRSCPEEQYWDPLLGTCMSCKTICNHQSQRTCAAFCRSLSCRKEQGKFYDHLLRDCISCA SICGQHPKQCAYFCENKLRS

**[0349]** In some embodiments, the reference TACI sequence is an extracellular domain region of TACI that consists essentially of only the CRD2 sequence and that is deleted in or lacks the entirety of the sequence of the CRD1 and substantially all of the stalk region. Although previous studies have shown that residues in the stalk region may contain a protease cleavage site, it was believed that at least the CRD1 and CRD2 was required for sufficient expression and/or binding activity of TACI for its cognate ligands. For example, international PCT publication No. WO2002/094852 demonstrated that a TACI molecule containing a CRD1 and CRD2, but in which the whole amino terminal region and a partial sequence of the stalk region was deleted, exhibited reduced protein degradation when expressed. Other studies showed that at least a portion of the N-terminal region before the CRD1 was necessary for sufficient binding activity of TACI for its cognate ligands, see e.g. international publication No. WO2008/154814, in which residues 13-118 or 13-108 of the TACI extracellular region were determined to be necessary for biological activity while minimizing degradation of TACI during expression. Surprisingly, it is found herein (e.g. Example 8) that a TACI extracellular region that consists essentially only of the CRD2 with a small portion of the stalk region exhibits substantially improved cognate binding activity compared to a longer TACI molecule containing both the CRD1 and CRD2.

**[0350]** In some embodiments, the BIM is a TACI polypeptide that is a portion of the TACI extracellular domain (ECD) region that contains the CRD2, with a deletion of the N-terminal region and CRD1 and deletion of one or more residues of the stalk portion of the TACI extracellular domain, e.g. relative to the sequence of amino acids set forth in SEQ ID NO:709. In some embodiments, the portion of the TACI extracellular domain that contains the CRD2 includes amino acid residues 71-104 corresponding to residues set forth in SEQ ID NO:709. In provided embodiments, the BIM of the immunomodulatory protein is a TACI polypeptide that contains deletion of N-terminal amino acid residues 1-66 corresponding to residues set forth in SEQ ID NO:709. In provided embodiments, the BIM of the immunomodulatory protein is a TACI polypeptide that contains deletion of N-terminal amino acid residues 1-67 corresponding to residues set forth in SEQ ID NO:709. In provided embodiments, the BIM of the immunomodulatory protein is a TACI polypeptide that contains deletion of N-terminal amino acid residues 1-68 corresponding to residues set forth in SEQ ID NO:709. In provided embodiments, the BIM of the immunomodulatory protein is a TACI polypeptide that contains deletion of N-terminal amino acid residues 1-69 corresponding to residues set forth in SEQ ID NO:709. In provided embodiments, the BIM of the immunomodulatory protein is a TACI polypeptide that contains deletion of N-terminal amino acid residues 1-70 corresponding to residues set forth in SEQ ID NO:709. In some of any such embodiments, the BIM of the immunomodulatory protein is a TACI polypeptide that lacks one or more contiguous C-terminal amino acid residues beginning at residue 105 and up to or including amino acid residue 166

corresponding to residues of the ECD sequence set forth in SEQ ID NO:709. In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61 or 62 of the ECD sequence is deleted.

**[0351]** In some embodiments, the BIM of an immunomodulatory protein provided herein is a TACI polypeptide with a sequence that contains an ECD portion having a contiguous sequence of amino acids of a TACI ECD that includes the CRD2 (e.g. residues 71-104 with reference to SEQ ID NO:709), but with a deletion of the N-terminal region and CRD1 and deletion of one or more residues of the stalk portion of the TACI extracellular domain, e.g. relative to the sequence of amino acids set forth in SEQ ID NO:709. For example, the TACI ECD portion can consist of the following with reference to amino acid residues set forth in SEQ ID NO:709: amino acid residues 67 to 118, amino acid residues 67 to 117, amino acid residues 67 to 116, amino acid residues 67 to 115, amino acid residues 67 to 114, amino acid residues 67 to 113, amino acid residues 67 to 112, amino acid residues 67 to 111, amino acid residues 67 to 110, amino acid residues 67 to 109, amino acid residues 67 to 108, amino acid residues 67 to 107, amino acid residues 67 to 106, amino acid residues 67 to 105, or amino acid residues 67 to 104. In some examples, the TACI ECD portion can consist of the following with reference to residues set forth in SEQ ID NO: 709: amino acid residues 68 to 118, amino acid residues 68 to 117, amino acid residues 68 to 116, amino acid residues 68 to 115, amino acid residues 68 to 114, amino acid residues 68 to 113, amino acid residues 68 to 112, amino acid residues 68 to 111, amino acid residues 68 to 110, amino acid residues 68 to 109, amino acid residues 68 to 108, amino acid residues 68 to 107, amino acid residues 68 to 106, amino acid residues 68 to 105, or amino acid residues 68 to 104. In some examples, the TACI ECD portion can consist of the following with reference to residues set forth in SEQ ID NO: 709: amino acid residues 69 to 118, amino acid residues 69 to 117, amino acid residues 69 to 116, amino acid residues 69 to 115, amino acid residues 69 to 114, amino acid residues 69 to 113, amino acid residues 69 to 112, amino acid residues 69 to 111, amino acid residues 69 to 110, amino acid residues 69 to 109, amino acid residues 69 to 108, amino acid residues 69 to 107, amino acid residues 69 to 106, amino acid residues 69 to 105, or amino acid residues 69 to 104. In some examples, the TACI ECD portion can consist of the following with reference to residues set forth in SEQ ID NO: 709: amino acid residues 70 to 118, amino acid residues 70 to 117, amino acid residues 70 to 116, amino acid residues 70 to 115, amino acid residues 70 to 114, amino acid residues 70 to 113, amino acid residues 70 to 112, amino acid residues 70 to 111, amino acid residues 70 to 110, amino acid residues 70 to 109, amino acid residues 70 to 108, amino acid residues 70 to 107, amino acid residues 70 to 106, amino acid residues 70 to 105, or amino acid residues 70 to 104. In some examples, the TACI ECD portion can consist of the following with reference to residues set forth in SEQ ID NO: 709: amino acid residues 71 to 118, amino acid residues 71 to 117, amino acid residues 71 to 116, amino acid residues 71 to 115, amino acid residues 71

to 114, amino acid residues 71 to 113, amino acid residues 71 to 112, amino acid residues 71 to 111, amino acid residues 71 to 110, amino acid residues 71 to 109, amino acid residues 71 to 108, amino acid residues 71 to 107, amino acid residues 71 to 106, amino acid residues 71 to 105, or amino acid residues 71 to 104. Any of the above TACI ECD sequences also can be a TACI reference sequence in accord with a TIM that is a variant TACI in the immunomodulatory proteins provided herein, in which such immunomodulatory proteins contain a variant TACI polypeptide that is modified by one or more amino acid modification (e.g. substitution) as described herein compared to such TACI reference sequence.

**[0352]** In particular, among a BIM in a provided immunomodulatory protein is a TACI ECD sequence that has or consists of the sequence set forth in SEQ ID NO:528 (encoded by the sequence of nucleotides set forth in SEQ ID NO:563. In some embodiments, the reference TACI sequence has or consists of the sequence set forth in SEQ ID NO:528, in which a provided variant TACI polypeptide is modified by one or more amino acid modification (e.g. substitution) as described herein compared to such reference TACI sequence.

**TACI ECD sequence (CRD2): SEQ ID NO:528**

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRS

**[0353]** Among BIMs in a provided immunomodulatory protein are variant TACI polypeptides. In some of any of the provided embodiments, the variant TACI sequence has the sequence of the reference (e.g. unmodified) TACI sequence, such as any described above, but additionally contains one more amino acid modifications, such as one or more amino acid substitutions. In particular, a BIM provided herein may be a variant TACI polypeptide containing at least one affinity-modified TD domain (e.g., CRD1 and/or CRD2) or a specific binding fragment thereof that contains one or more amino acid substitutions in a TD domain of a reference (e.g., unmodified or wild-type) TACI polypeptide, such that the variant TACI polypeptide exhibits altered (e.g. increased) binding activity or affinity for one or both of APRIL or BAFF compared to the reference (e.g., unmodified or wild-type) TACI polypeptide. In some embodiments, a BIM is a variant TACI polypeptide that has a binding affinity for APRIL and/or BAFF that differs from that of a reference (e.g., unmodified or wild-type) TACI polypeptide control sequence as determined by, for example, solid-phase ELISA immunoassays, flow cytometry or Biacore assays. Binding affinities for each of the cognate binding partners are independent; that is, in some embodiments, a variant TACI polypeptide has an increased binding affinity for one or both APRIL and BAFF, and a decreased or unchanged binding affinity for the other of APRIL or BAFF, relative to a reference (e.g., unmodified or wild-type) TACI polypeptide.

**[0354]** In some embodiments, the BIM is a variant TACI polypeptide that has an increased binding affinity for BAFF, relative to the reference (unmodified or wild-type) TACI polypeptide. In some embodiments, the BIM is a variant TACI polypeptide that has an increased binding affinity for APRIL relative to the reference (unmodified or wild-type) TACI polypeptide. In some embodiments, the BIM is

a variant TACI polypeptide has an increased binding affinity for APRIL and BAFF relative to the reference (unmodified or wild-type) TACI polypeptide. The cognate ligands BAFF and/or APRIL can be a mammalian protein, such as a human protein or a murine protein. In some embodiments, a BIM that is a variant TACI polypeptide with increased or greater binding affinity to APRIL and/or BAFF will have an increase in binding affinity relative to the reference (e.g., unmodified or wild-type) TACI polypeptide control of at least about 5%, such as at least about 10%, 15%, 20%, 25%, 35%, or 50%. In some embodiments, the increase in binding affinity relative to the reference (e.g., unmodified or wild-type) TACI polypeptide is more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold 40-fold or 50-fold. In any of the examples, the reference (e.g., unmodified or wild-type) TACI polypeptide has the same sequence as the variant TACI polypeptide except that it does not contain the one or more amino acid modifications (e.g., substitutions).

**[0355]** In some embodiments, the equilibrium dissociation constant ($K_d$) of any of the foregoing embodiments to BAFF can be less than $1x10^{-5}$ M, $1x10^{-6}$ M, $1x10^{-7}$ M, $1x10^{-8}$ M, $1x10^{-9}$ M, $1x10^{-10}$ M or $1x10^{-11}$M, or $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to BAFF is less than at or about $1x10^{-9}$ M, $1x10^{-10}$ M or $1x10^{-11}$M, or $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to BAFF is between $1x10^{-9}$ M and at or about $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to BAFF is at or about $1x10^{-9}$ M, at or about $2x10^{-9}$ M, at or about $4x10^{-9}$ M, at or about $6x10^{-9}$ M, at or about $8x10^{-9}$ M, at or about $1x10^{-10}$ M, at or about $2x10^{-10}$ M, at or about $4x10^{-10}$ M, at or about $6x10^{-10}$ M, at or about $8x10^{-10}$ M, at or about $1x10^{-11}$ M, at or about $2x10^{-11}$ M, at or about $4x10^{-11}$ M, at or about $6x10^{-11}$ M, at or about $8x10^{-11}$ M, or at or about $1x10^{-12}$ M, or any value between any of the foregoing. In some embodiments, a BIM in a provided embodiment is a variant TACI polypeptide as described above and the $K_d$ to BAFF is decreased (higher binding affinity) by greater than or greater than about 1.5-fold, such as greater than or about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold or more.

**[0356]** In some embodiments, the equilibrium dissociation constant ($K_d$) of any of the foregoing embodiments to APRIL can be less than $1x10^{-5}$ M, $1x10^{-6}$ M, $1x10^{-7}$ M, $1x10^{-8}$ M, $1x10^{-9}$ M, $1x10^{-10}$ M or $1x10^{-11}$M, or $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to APRIL is less than at or about $1x10^{-9}$ M, $1x10^{-10}$ M or $1x10^{-11}$M, or $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to APRIL is between $1x10^{-9}$ M and at or about $1x10^{-12}$ M. In some embodiments, the $K_d$ of any of the foregoing embodiments to APRIL is at or about $1x10^{-9}$ M, at or about $2x10^{-9}$ M, at or about $4x10^{-9}$ M, at or about $6x10^{-9}$ M, at or about $8x10^{-9}$ M, at or about $1x10^{-10}$ M, at or about $2x10^{-10}$ M, at or about $4x10^{-10}$ M, at or about $6x10^{-10}$ M, at or about $8x10^{-10}$ M, at or about $1x10^{-11}$ M, at or about $2x10^{-11}$ M, at or about $4x10^{-11}$ M, at or about $6x10^{-11}$ M, at or about $8x10^{-11}$ M, or at or about $1x10^{-12}$ M, or any value between any of the foregoing. In some embodiments, a BIM of a provided embodiment is a variant TACI polypeptide as described above and the $K_d$ to APRIL is decreased (higher

binding affinity) by greater than or greater than about 1.5-fold, such as greater than or about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold or more.

**[0357]** The reference (e.g., unmodified or wild-type) TACI sequence does not necessarily have to be used as a starting composition to generate a BIM that is a variant TACI polypeptide described herein. Therefore, use of the term "modification", such as "substitution" does not imply that the present embodiments are limited to a particular method of making variant TACI polypeptides or immunomodulatory proteins containing the same. Variant TACI polypeptides can be made, for example, by *de novo* peptide synthesis and thus does not necessarily require a modification, such as a "substitution", in the sense of altering a codon to encode for the modification, e.g. substitution. This principle also extends to the terms "addition" and "deletion" of an amino acid residue which likewise do not imply a particular method of making. The means by which the variant TACI polypeptides are designed or created is not limited to any particular method. In some embodiments, however, a reference (e.g., unmodified or wild-type) TACI encoding nucleic acid is mutagenized from reference (e.g., unmodified or wild-type) TACI genetic material and screened for desired specific binding affinity or other functional activity. In some embodiments, a variant TACI polypeptide is synthesized *de novo* utilizing protein or nucleic acid sequences available at any number of publicly available databases and then subsequently screened. The National Center for Biotechnology Information provides such information and its website is publicly accessible via the internet as is the UniProtKB database as discussed previously.

**[0358]** Unless stated otherwise, as indicated throughout the present disclosure, reference to an amino acid modification (s) of a BIM that is a variant TACI polypeptide are designated by amino acid position number corresponding to the numbering of positions of the reference ECD sequence set forth in SEQ ID NO:709. It is within the level of a skilled artisan to identify the corresponding position of a modification, e.g. amino acid substitution, in an TACI polypeptide, including portion thereof containing TD (e.g. CRD1 and/or CRD2) thereof, such as by alignment of a reference sequence (e.g. SEQ ID NO:516 or 528) with SEQ ID NO:709. An alignment identifying corresponding residues is exemplified in FIG. 17A. In the listing of modifications throughout this disclosure, the amino acid position is indicated in the middle, with the corresponding reference (e.g. unmodified or wild-type) amino acid listed before the number and the identified variant amino acid substitution listed after the number. If the modification is a deletion of the position a "del" is indicated and if the modification is an insertion at the position an "ins" is indicated. In some cases, an insertion is listed with the amino acid position indicated in the middle, with the corresponding reference amino acid listed before and after the number and the identified variant amino acid insertion listed after the unmodified (e.g. wild-type) amino acid.

**[0359]** In some embodiments, a BIM is a variant TACI polypeptide that has one or more amino acid modification, e.g. substitution in a reference (e.g., unmodified or wild-type) TACI sequence, such as any

as described. The one or more amino acid modification, e.g. substitution, can be in the ectodomain (extracellular domain) of the reference (e.g., unmodified or wild-type) TACI sequence. In some embodiments, the one or more amino acid modification, e.g. substitution is in the CRD1 domain or specific binding fragment thereof. In some embodiments, the one or more amino acid modification, e.g. substitution is in the CRD2 domain or specific binding fragment thereof. In some embodiments of the variant TACI polypeptide, some of the one or more amino acid modification, e.g. substitution is in the CRD1 domain or a specific binding fragment thereof, and some of the one or more amino acid modification, e.g. substitution are in the CRD2 domain or a specific binding fragment thereof.

**[0360]** In some embodiments, a BIM that is a variant TACI polypeptide has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modification(s), e.g. substitution. The modification, e.g. substitution can be in the CRD1 domain or the CRD2 domain. In some embodiments, a BIM that is a variant TACI polypeptide has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions in the CRD1 domain or specific binding fragment thereof. In some embodiments, a BIM that is a variant TACI polypeptide has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions in the CRD2 domain or specific binding fragment thereof. In some embodiments, the variant TACI polypeptide containing the one or more amino acid modifications (e.g. amino acid substitutions) as described has at least about 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the reference (e.g., unmodified or wild-type) TACI polypeptide or specific binding fragment thereof, such as with the amino acid sequence of SEQ ID NO: 516, 528 or 709. In some embodiments, the variant TACI polypeptide containing the one or more amino acid modifications (e.g. amino acid substitutions) as described has at least about 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 709. In some embodiments, the variant TACI polypeptide containing the one or more amino acid modifications (e.g. amino acid substitutions) as described has at least about 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 516. In some embodiments, the variant TACI polypeptide containing the one or more amino acid modifications (e.g. amino acid substitutions) as described has at least about 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 528.

**[0361]** In some embodiments, a BIM that is a variant TACI polypeptide has one or more amino acid modification, e.g. substitution in a reference TACI polypeptide or specific binding fragment there of corresponding to position(s) 40, 59, 60, 61, 74, 75, 76, 77, 78, 79, 82, 83, 84, 85, 86, 87, 88, 92, 95, 97, 98, 99, 101, 102 and 103 with reference to numbering of SEQ ID NO:709. In some embodiments, a BIM that is a variant TACI polypeptide has one or more amino acid modification, e.g. substitution selected

from W40R, Q59R, R60G, T61P, E74V, Q75E, Q75R, G76S, K77E, F78Y, Y79F, L82H, L82P, L83S, R84G, R84L, R84Q, D85E, D85V, C86Y, I87L, I87M, S88N, I92V, Q95R, P97S, K98T, Q99E, A101D, Y102D, F103S, F103V, F103Y, or a conservative amino acid substitution thereof. In some embodiments, the reference TACI polypeptide includes the CRD1 domain or CRD2 domain, for example the reference TACI polypeptide is set forth in SEQ ID NO: 516 or SEQ ID NO:709.

**[0362]** In some embodiments, the amino acid substitutions are in the CRD2 domain only. In some embodiments, a BIM that is a variant TACI polypeptide has one or more amino acid modification, e.g. substitution in a reference TACI polypeptide or specific binding fragment there of corresponding to position(s) 74, 75, 76, 77, 78, 79, 82, 83, 84, 85, 86, 87, 88, 92, 95, 97, 98, 99, 101, 102 and 103 with reference to numbering of SEQ ID NO:709. In some embodiments, a BIM that is a variant TACI polypeptide has one or more amino acid modification, e.g. substitution selected from E74V, Q75E, Q75R, G76S, K77E, F78Y, Y79F, L82H, L82P, L83S, R84G, R84L, R84Q, D85E, D85V, C86Y, I87L, I87M, S88N, I92V, Q95R, P97S, K98T, Q99E, A101D, Y102D, F103S, F103V, F103Y, or a conservative amino acid substitution thereof. In some embodiments, among the CRD domains, the reference TACI polypeptide includes only the CRD2 domain but lacks the CRD1 domain, for example the reference TACI polypeptide is set forth in SEQ ID NO: 528. Accordingly, in some embodiments, a BIM that is a variant TACI polypeptide includes a portion of the ECD sequence of a TACI polypeptide that includes the CRD2 domain but lacks the CRD1 domain.

**[0363]** A conservative amino acid modification, e.g. substitution is any amino acid that falls in the same class of amino acids as the substituted amino acids, other than the reference (e.g., unmodified) or wild-type amino acid. The classes of amino acids are aliphatic (glycine, alanine, valine, leucine, and isoleucine), hydroxyl or sulfur-containing (serine, cysteine, threonine, and methionine), cyclic (proline), aromatic (phenylalanine, tyrosine, tryptophan), basic (histidine, lysine, and arginine), and acidic/amide (aspartate, glutamate, asparagine, and glutamine).

**[0364]** In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution at position 75 with reference to numbering of SEQ ID NO:709. In some embodiments, the amino acid substitution at position 75 confers increased binding to BAFF or APRIL compared to the reference (e.g. wildtype or unmodified) TACI polypeptide not containing the amino acid substitution. In some embodiments, the substituted amino acid is an acidic amino acid or amide, such as to a different acidic amino acid or amide compared to the reference (e.g. wildtype or unmodified) TACI polypeptide. In some embodiments, the substituted amino acid at position 75 is a glutamic acid (Glu, E). In some embodiments, the substituted amino acid at position 75 is an asparatic acid (Asp, D). In some embodiments, the substituted amino acid at position 75 is an asparagine (Asn, N). In some embodiments, the substituted amino acid at position 75 is a glutamine (Gln, Q).

**[0365]** In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution at position 77 with reference to numbering of SEQ ID NO:709. In some embodiments, the amino acid substitution at position 77 confers increased binding to BAFF or APRIL compared to the reference (e.g. wildtype or unmodified) TACI polypeptide not containing the amino acid substitution. In some embodiments, the substituted amino acid at position 77 is an acidic amino acid or amide. In some embodiments, the substituted amino acid at position 77 is a glutamic acid (Glu, E). In some embodiments, the substituted amino acid at position 77 is an asparatic acid (Asp, D). In some embodiments, the substituted amino acid at position 77 is an asparagine (Asn, N). In some embodiments, the substituted amino acid at position 77 is a glutamine (Gln, Q).

**[0366]** In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution at position 78 with reference to numbering of SEQ ID NO:709. In some embodiments, the amino acid substitution at position 78 confers increased binding to BAFF or APRIL compared to the reference (e.g. wildtype or unmodified) TACI polypeptide not containing the amino acid substitution. In some embodiments, the substituted amino acid at position 78 is an aromatic amino acid, such as to a different aromatic amino acid compared to the reference (e.g. wildtype or unmodified) TACI polypeptide. In some embodiments, the substituted amino acid at position 78 is a phenyalanine (Phe, F). In some embodiments, the substituted amino acid at position 78 is a tyrosine (Tyr, Y). In some embodiments, the substituted amino acid at position 78is a tryptophan (Trp, W).

**[0367]** In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution at position 84 with reference to numbering of SEQ ID NO:709. In some embodiments, the amino acid substitution at position 84 confers increased binding to BAFF or APRIL compared to the reference (e.g. wildtype or unmodified) TACI polypeptide not containing the amino acid substitution. In some embodiments, the substituted amino acid at position 84 is an acidic amino acid or amide. In some embodiments, the substituted amino acid at position 84 is a glutamic acid (Glu, E). In some embodiments, the substituted amino acid at position 84 is an asparatic acid (Asp, D). In some embodiments, the substituted amino acid at position 84 is an asparagine (Asn, N). In some embodiments, the substituted amino acid at position 84 is a glutamine (Gln, Q).

**[0368]** In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution at position 102 with reference to numbering of SEQ ID NO:709. In some embodiments, the amino acid substitution at position 102 confers increased binding to BAFF or APRIL compared to the reference (e.g. wildtype or unmodified) TACI polypeptide not containing the amino acid substitution. In some embodiments, the substituted amino acid at position 102 is an acidic amino acid or amide. In some embodiments, the substituted amino acid at position 102 is a glutamic acid (Glu, E). In some embodiments, the substituted amino acid at position 102 is an asparatic acid (Asp, D). In some

embodiments, the substituted amino acid at position 102 is an asparagine (Asn, N). In some embodiments, the substituted amino acid at position 102 is a glutamine (Gln, Q).

**[0369]** In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution E74V. In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution Q75E.In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution K77E. In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution F78Y. In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution Y79F. In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution L82H. In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution L82P. In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution R84G. In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution R84L. In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution R84Q. In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution D85V. In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution C86Y. In some embodiments, a BIM that is a variant TACI polypeptide includes at least one amino acid substitution Y102D. In some embodiments, a BIM that is a variant TACI polypeptide contains two or more amino acid substitutions of any two or more of the foregoing. In some embodiments, a BIM that is a variant TACI polypeptide includes one or more amino acid substitution that is a conservative amino acid substitution of any of the foregoing. In provided embodiments, a BIM that is a variant TACI polypeptide includes the at least one amino acid substitution in any reference TACI polypeptide sequence as described. In some embodiments, the at least one amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 516. In some embodiments, the at least one amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 528. In some embodiments, the at least one amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 718. In some embodiments, the at least one amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 719.

**[0370]** In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution E74V. In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution Q75E.In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution K77E. In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution F78Y. In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution Y79F. In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution L82H. In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution L82P. In some embodiments, a BIM that is a

variant TACI polypeptide includes the amino acid substitution R84G. In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution R84L. In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution R84Q. In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution D85V. In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution C86Y. In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution Y102D. In some embodiments, a BIM that is a variant TACI polypeptide contains two or more amino acid substitutions of any two or more of the foregoing. In some embodiments, a BIM that is a variant TACI polypeptide includes one or more of amino acid substitution that is a conservative amino acid substitution of any of the foregoing. In provided embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitution in any reference TACI polypeptide sequence as described. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 516. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 528. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 718. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 719.In some embodiments, the amino acid substitutions are D85E/K98T. In some embodiments, the amino acid substitutions are I87L/K98T. In some embodiments, the amino acid substitutions are R60G/Q75E/L82P. In some embodiments, the amino acid substitutions are R60G/C86Y. In some embodiments, the amino acid substitutions are W40R/L82P/F103Y. In some embodiments, the amino acid substitutions are W40R/Q59R/T61P/K98T. In some embodiments, the amino acid substitutions are L82P/I87L. In some embodiments, the amino acid substitutions are G76S/P97S. In some embodiments, the amino acid substitutions are K77E/R84L/F103Y. In some embodiments, the amino acid substitutions are Y79F/Q99E. In some embodiments, the amino acid substitutions are L83S/F103S. In some embodiments, the amino acid substitutions are K77E/R84Q. In some embodiments, the amino acid substitutions are K77E/A101D. In some embodiments, the amino acid substitutions are K77E/F78Y/Y102D. In some embodiments, the amino acid substitutions are Q75E/R84Q. In some embodiments, the amino acid substitutions are Q75R/R84G/I92V. In some embodiments, the amino acid substitutions are K77E/A101D/Y102D. In some embodiments, the amino acid substitutions are R84Q/S88N/A101D. In some embodiments, the amino acid substitutions are R84Q/F103V. In some embodiments, the amino acid substitutions are K77E/Q95R/A101D. In some embodiments, the amino acid substitutions are I87M/A101D. In provided embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitutions in any reference TACI polypeptide sequence as described. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 516. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 528. In some embodiments, the amino acid

substitution is in the reference TACI sequence set forth in SEQ ID NO: 718. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 719.

**[0371]** In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitutions K77E and F78Y (K77E/F78Y). In provided embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitutions in any reference TACI polypeptide sequence as described. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 516. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 528. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 718. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 719.

**[0372]** In some embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitutions K77E and Y102D (K77E/Y102D). In provided embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitutions in any reference TACI polypeptide sequence as described. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 516. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 528. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 718. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 719.

**[0373]** In some embodiments, a BIM that is a variant TACI polypeptide contains the amino acid substitutions F78Y and Y102D (F78Y/Y012D). In provided embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitutions in any reference TACI polypeptide sequence as described. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 516. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 528. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 718. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 719.

**[0374]** In some embodiments a BIM that is a variant TACI polypeptide contains the amino acid substations K77E, F78Y and Y102D (K77E/F78Y/Y102D). In provided embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitutions in any reference TACI polypeptide sequence as described. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 516. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 528. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 718. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 719.

**[0375]** In some embodiments, a BIM that is a variant TACI polypeptide contains the amino acid substitutions Q75E/R84Q. In provided embodiments, a BIM that is a variant TACI polypeptide includes the amino acid substitutions in any reference TACI polypeptide sequence as described. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 516. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 528. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 718. In some embodiments, the amino acid substitution is in the reference TACI sequence set forth in SEQ ID NO: 719.

**[0376]** In some embodiments, a BIM that is a variant TACI polypeptide comprises any of the mutations listed in Table 2. Table 2 also provides exemplary sequences by reference to SEQ ID NO of the reference (e.g., unmodified) TACI polypeptide, and exemplary variant TACI polypeptides. As indicated, the exact locus or residues corresponding to a given domain can vary, such as depending on the methods used to identify or classify the domain. Also, in some cases, adjacent N- and/or C-terminal amino acids of a given domain (e.g. CRD) also can be included in a sequence of a BIM that is a variant TACI polypeptide, such as to ensure proper folding of the domain when expressed. Thus, it is understood that the exemplification of the SEQ ID NOSs in Table 2 is not to be construed as limiting. For example, the particular domain, such as the ECD domain or a portion thereof containing the CRD1/CRD2 or CRD2 only, of a variant TACI polypeptide can be several amino acids longer or shorter, such as 1-10, e.g., 1, 2, 3, 4, 5, 6 or 7 amino acids longer or shorter, than the sequence of amino acids set forth in the respective SEQ ID NO.

**[0377]** In some embodiments, a BIM that is a variant TACI polypeptide comprises any of the mutations (amino acid substitutions) listed in Table 2. In some examples, the mutations (amino acid substitutions) are made in a reference TACI containing the sequence of amino acids set forth in SEQ ID NO: 709. In some examples, the mutations (amino acid substitutions) are made a reference TACI that contains the CRD1 and CRD2 domain of TACI, for example as set forth in SEQ ID NO: 516. In some examples, the mutations (amino acid substitutions) are made in a reference TACI that is further truncated by deletion of N-terminal and C-terminal amino acid residues to retain the CRD2, for example as set forth in SEQ ID NO: 528.

**[0378]** The use of the term “modification”, such as “substitution” or “mutation,” does not imply that the present embodiments are limited to a particular method of making the immunomodulatory proteins. A BIM that is a variant TACI polypeptide can be made, for example, by *de novo* peptide synthesis and thus does not necessarily require a modification, such as a “substitution” in the sense of altering a codon to encode for the modification, e.g. substitution. This principle also extends to the terms “addition” and “deletion” of an amino acid residue which likewise do not imply a particular method of making. The means by which the vTDs are designed or created is not limited to any particular method.

In some embodiments, however, a wild-type or unmodified TD encoding nucleic acid is mutagenized from wild-type or unmodified TD genetic material and screened for desired specific binding activity, e.g. binding affinity, and/or alteration of NF-κB modulation or other functional activity. In some embodiments, a vTD is synthesized *de novo* utilizing protein or nucleic acid sequences available at any number of publicly available databases and then subsequently screened. The National Center for Biotechnology Information provides such information and its website is publicly accessible via the internet as is the UniProtKB database.

**[0379]** In some embodiments, a BIM that is a variant TACI polypeptide comprises an extracellular domain (ECD) sequences containing a CRD1 and CRD2, such as a variant TACI polypeptide set forth in any one of SEQ ID NOS: 517-527, 536, 537, 682-701. In some embodiments, a BIM that is a variant TACI polypeptide comprises a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 517-527, 536, 537, 682-701, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) TACI. In some embodiments, a BIM that is a variant TACI polypeptide comprises a specific binding fragment of any one of SEQ ID NOS: 517-527, 536, 537, 682-701, in which the specific binding fragment binds BAFF, APRIL or a BAFF/APRIL heterotrimer, and contains a contiguous sequence therein that contains the amino acid modification(s), e.g. substitution (s) therein not present in the reference (e.g., unmodified or wild-type) TACI.

**[0380]** In some embodiments, a BIM that is a variant TACI polypeptide consists or consists essentially of a variant TACI extracellular domain (ECD) sequences set forth in any one of SEQ ID NOS: 517-527, 536, 537, 682-701. In some embodiments, a BIM that is a variant TACI polypeptide consists or consists essentially of a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 517-527, 536, 537, 682-701, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) TACI. In some embodiments, a BIM that is a variant TACI polypeptide consists or consists essentially of a specific binding fragment of any one of SEQ ID NOS: 517-527, 536, 537, 682-701, in which the specific binding fragment binds BAFF, APRIL or an APRIL/BAFF heterotrimer and contains a contiguous sequence therein that contains the amino acid modification(s), e.g. substitution (s) therein not present in the reference (e.g., unmodified or wild-type) TACI.

**[0381]** In some embodiments, a BIM that is a variant TACI polypeptide comprises an extracellular domain (ECD) sequences containing a CRD2 but lacking the CRD1 of a reference TACI polypeptide, such as a variant TACI polypeptide set forth in any one of SEQ ID NOS: 529-535, 538-550, 673-681. In

some embodiments, a BIM that is a variant TACI polypeptide comprises a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 529-535, 538-550, 673-681, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) TACI. In some embodiments, a BIM that is a variant TACI polypeptide comprises a specific binding fragment of any one of SEQ ID NOS: 529-535, 538-550, 673-681, in which the specific binding fragment binds BAFF, APRIL or a BAFF/APRIL heterotrimer, and contains a contiguous sequence therein that contains the amino acid modification(s), e.g. substitution (s) therein not present in the reference (e.g., unmodified or wild-type) TACI.

**[0382]** In some embodiments, a BIM that is a variant TACI polypeptide consists or consists essentially of the sequence set forth in any one of SEQ ID NOS: 529-535, 538-550, 673-681. In some embodiments, a BIM that is a variant TACI polypeptide consists or consists essentially of a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 529-535, 538-550, 673-681, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) TACI. In some embodiments, a BIM that is a variant TACI polypeptide consists or consists essentially of a specific binding fragment of any one of SEQ ID NOS: 529-535, 538-550, 673-681, in which the specific binding fragment binds BAFF, APRIL or a BAFF/APRIL heterotrimer, and contains a contiguous sequence therein that contains the amino acid modification(s), e.g. substitution (s) therein not present in the reference (e.g., unmodified or wild-type) TACI.

**[0383]** In some embodiments, a BIM that is a variant TACI polypeptide is one in which the variant TACI polypeptide comprises the sequence set forth in SEQ ID NO:535. In some embodiments, the variant TACI polypeptide consists essentially of the sequence set forth in SEQ ID NO:535. In some embodiments, the variant TACI polypeptide consists of the sequence set forth in SEQ ID NO:535.

**[0384]** In some embodiments, a BIM that is a variant TACI polypeptide is one in which the variant TACI polypeptide comprises the sequence set forth in SEQ ID NO:541. In some embodiments, the variant TACI polypeptide consists essentially of the sequence set forth in SEQ ID NO:541. In some embodiments, the variant TACI polypeptide consists of the sequence set forth in SEQ ID NO:541.

**[0385]** In some embodiments, a BIM that is a variant TACI polypeptide is one in which the variant TACI polypeptide comprises the sequence set forth in SEQ ID NO:542. In some embodiments, the variant TACI polypeptide consists essentially of the sequence set forth in SEQ ID NO:542. In some embodiments, the variant TACI polypeptide consists of the sequence set forth in SEQ ID NO:542.

**[0386]** In some embodiments, a BIM that is a variant TACI polypeptide is one in which the variant TACI polypeptide comprises the sequence set forth in SEQ ID NO:688. In some embodiments, the variant TACI polypeptide consists essentially of the sequence set forth in SEQ ID NO:688. In some embodiments, the variant TACI polypeptide consists of the sequence set forth in SEQ ID NO:688.

**[0387]** In some embodiments, a BIM that is a variant TACI polypeptide is one in which the variant TACI polypeptide is encoded by a sequence of nucleotides set forth in any of SEQ ID NOS: 552-562, 571 or 572. In some embodiments, the variant TACI polypeptide is encoded by a sequence of nucleotides that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 552-562, 571 or 572, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) TACI.

**[0388]** In some embodiments, a BIM that is a variant TACI polypeptide is one in which the variant TACI polypeptide is encoded by a sequence of nucleotides set forth in any of SEQ ID NOS: 564-570 or 573-585. In some embodiments, the variant TACI polypeptide is encoded by a sequence of nucleotides that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 564-570 or 573-585, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) TACI.

**TABLE 2: Exemplary variant TACI BIMs**

| Name | Mutation(s) | ECD (CRD1/CRD2) | | ECD (CRD2) | |
|---|---|---|---|---|---|
| | | AA SEQ ID NO | NT SEQ ID NO | AA SEQ ID NO | NT SEQ ID NO |
| 516 (WT) TACI CRD1/CRD2<br>528 (WT) TACI CRD2 | Wild-type | 516 | 551 | 528 | 563 |
| 517 TACI CRD1/CRD2<br>673 TACI CRD2 | L82P | 517 | 552 | 673 | |
| 518 TACI CRD1/CRD2<br>674 TACI CRD2 | D85E, K98T | 518 | 553 | 674 | |
| 519 TACI CRD1/CRD2<br>675 TACI CRD2 | I87L, K98T | 519 | 554 | 675 | |
| 520 TACI CRD1/CRD2 | R60G, Q75E, L82P | 520 | 555 | | |
| 521 TACI CRD1/CRD2 | R60G, C86Y | 521 | 556 | | |

**TABLE 2: Exemplary variant TACI BIMs**

| Name | Mutation(s) | ECD (CRD1/CRD2) | | ECD (CRD2) | |
|---|---|---|---|---|---|
| | | AA SEQ ID NO | NT SEQ ID NO | AA SEQ ID NO | NT SEQ ID NO |
| 522 TACI CRD1/CRD2 676 TACI CRD2 | A101D | 522 | 557 | 676 | |
| 523 TACI CRD1/CRD2 677 TACI CRD2 | C86Y | 523 | 558 | 677 | |
| 524 TACI CRD1/CRD2 | W40R, L82P, F103Y | 524 | 559 | | |
| 525 TACI CRD1/CRD2 | W40R, Q59R, T61P, K98T | 525 | 560 | | |
| 526 TACI CRD1/CRD2 678 TACI CRD2 | L82P, I87L | 526 | 561 | 678 | |
| 527 TACI CRD1/CRD2 679 TACI CRD2 | G76S, P97S | 527 | 562 | 679 | |
| 682 TACI CRD1/CRD2 529 TACI CRD2 | D85V | 682 | | 529 | 564 |
| 683 TACI CRD1/CRD2 530 TACI CRD2 | E74V | 683 | | 530 | 565 |
| 684 TACI CRD1/CRD2 531 TACI CRD2 | R84L | 684 | | 531 | 566 |
| 685 TACI CRD1/CRD2 532 TACI CRD2 | K77E, R84L, F103Y | 685 | | 532 | 567 |
| 686 TACI CRD1/CRD2 533 TACI CRD2 | Y79F, Q99E | 686 | | 533 | 568 |
| 687 TACI CRD1/CRD2 534 TACI CRD2 | Y79F | 687 | | 534 | 569 |
| 536 TACI CRD1/CRD2 680 TACI CRD2 | R84G | 688 | | 535 | 570 |
| 536 TACI CRD1/CRD2 680 TACI CRD2 | L83S, F103S | 536 | 571 | 680 | |
| 537 TACI CRD1/CRD2 681 TACI CRD2 | L82H | 537 | 572 | 681 | |
| 689 TACI CRD1/CRD2 538 TACI CRD2 | A101D | 689 | | 538 | 573 |
| 690 TACI CRD1/CRD2 539 TACI CRD2 | K77E, R84Q | 690 | | 539 | 574 |
| 691 TACI CRD1/CRD2 540 TACI CRD2 | K77E, A101D | 691 | | 540 | 575 |
| 692 TACI CRD1/CRD2 541 TACI CRD2 | K77E, F78Y, Y102D | 692 | | 541 | 576 |
| 693 TACI CRD1/CRD2 542 TACI CRD2 | Q75E, R84Q | 693 | | 542 | 577 |
| 694 TACI CRD1/CRD2 543 TACI CRD2 | Q75R, R84G, I92V | 694 | | 543 | 578 |

**TABLE 2: Exemplary variant TACI BIMs**

| Name | Mutation(s) | ECD (CRD1/CRD2) | | ECD (CRD2) | |
|---|---|---|---|---|---|
| | | AA SEQ ID NO | NT SEQ ID NO | AA SEQ ID NO | NT SEQ ID NO |
| 695 TACI CRD1/CRD2 544 TACI CRD2 | K77E, A101D, Y102D | 695 | | 544 | 579 |
| 696 TACI CRD1/CRD2 545 TACI CRD2 | R84Q | 696 | | 545 | 580 |
| 697 TACI CRD1/CRD2 546 TACI CRD2 | R84Q, S88N, A101D | 697 | | 546 | 581 |
| 698 TACI CRD1/CRD2 547 TACI CRD2 | K77E | 698 | | 547 | 582 |
| 699 TACI CRD1/CRD2 548 TACI CRD2 | R84Q, F103V | 699 | | 548 | 583 |
| 700 TACI CRD1/CRD2 549 TACI CRD2 | K77E, Q95R, A101D | 700 | | 549 | 584 |
| 701 TACI CRD1/CRD2 550 TACI CRD2 | I87M, A101D | 701 | | 550 | 585 |
| 769 TACI CRD2 | Q75E | | | 769 | |
| 770 TACI CRD2 | Q75E, K77E | | | 770 | |
| 771 TACI CRD2 | Q75E, F78Y | | | 771 | |
| 772 TACI CRD2 | Q75E, A101D | | | 772 | |
| 773 TACI CRD2 | Q75E, Y102D | | | 773 | |
| 774 TACI CRD2 | K77E, F78Y, R84Q | | | 774 | |
| 775 TACI CRD2 | F78Y | | | 775 | |
| 776 TACI CRD2 | F78Y, R84Q | | | 776 | |
| 777 TACI CRD2 | F78Y, A101D | | | 777 | |
| 778 TACI CRD2 | F78Y, Y102D | | | 778 | |
| 779 TACI CRD2 | R84Q, A101D | | | 779 | |
| 780 TACI CRD2 | R84Q, Y102D | | | 780 | |
| 781 TACI CRD2 | A101D, Y102D | | | 781 | |
| 792 TACI CRD2 | Y102D | | | 792 | |

| TABLE 2: Exemplary variant TACI BIMs | | | | | |
|---|---|---|---|---|---|
| Name | Mutation(s) | ECD (CRD1/CRD2) | | ECD (CRD2) | |
| | | AA SEQ ID NO | NT SEQ ID NO | AA SEQ ID NO | NT SEQ ID NO |
| 793 TACI CRD2 | K77E, F78Y | | | 793 | |
| 794 TACI CRD2 | K77E, Y102D | | | 794 | |

**[0389]** In some embodiments, the BIM is or contains a wild-type or unmodified ECD of TACI or a specific binding portion or fragment thereof containing at least one TD (e.g. at least one CRD, such as the CRD1 and/or CRD2) that binds to APRIL, BAFF and/or an APRIL/BAFF heterotrimer. In some embodiments, the BIM contains the ECD sequence set forth in SEQ ID NO: 709, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:709 and that binds to APRIL, BAFF or an APRIL/BAFF heterotirmer, or (iii) is a fragment or portion of (i) or (ii) containing a CRD1 and/or CRD2, in which the portion binds to APRIL, BAFF or an APRI/BAFF heterotrimer. In some embodiments, the BIM is a TACI sequence that consists or consists essentially of the sequence set forth in SEQ ID NO: 709. In some embodiments, the BIM is a TACI sequence containing residues 2-166 of SEQ ID NO:709 that lacks the N-terminal methionine as set forth in SEQ ID NO: 709. In some embodiments, the BIM is a TACI sequence that consists or consists essentially of the sequence set forth as amino acids 2-166 of SEQ ID NO: 709.

**[0390]** In some embodiments, the BIM is or contains a binding portion of the wild-type or unmodified ECD of TACI or a specific binding portion or fragment thereof that contains the CRD1 and CRD2 of TACI and that binds to APRIL, BAFF and/or an APRIL/BAFF heterotrimer. In some embodiments, the BIM contains the sequence set forth in SEQ ID NO: 719, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:719 and that binds to APRIL, BAFF or an APRIL/BAFF heterotrimer, or (iii) is a fragment or portion of (i) or (ii) containing a portion of the CRD1 and/or CRD2, in which the portion binds to APRIL, BAFF or an APRI/BAFF heterotrimer. In some embodiments, the BIM comprises the sequence set forth in SEQ ID NO: 719. In some embodiments, the BIM is a TACI sequence that consists or consists essentially of the sequence set forth in SEQ ID NO: 719. In some embodiments, the BIM contains the sequence set forth in SEQ ID NO: 718, (ii) a sequence

of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:718 and that binds to APRIL, BAFF or an APRIL/BAFF heterotrimer, or (iii) is a fragment or portion of (i) or (ii) containing a portion of the CRD1 and/or CRD2, in which the portion binds to APRIL, BAFF or an APRI/BAFF heterotrimer. In some embodiments, the BIM comprises the sequence set forth in SEQ ID NO: 718. In some embodiments, the BIM is a TACI sequence that consists or consists essentially of the sequence set forth in SEQ ID NO: 718. In some embodiments, the BIM contains the sequence set forth in SEQ ID NO: 516, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:516 and that binds to APRIL, BAFF or an APRIL/BAFF heterotrimer, or (iii) is a fragment or portion of (i) or (ii) containing a portion of the CRD1 and/or CRD2, in which the portion binds to APRIL, BAFF or an APRI/BAFF heterotrimer. In some embodiments, the BIM comprises the sequence set forth in SEQ ID NO: 516. In some embodiments, the BIM is a TACI sequence that consists or consists essentially of the sequence set forth in SEQ ID NO: 516.

**[0391]** In some embodiments, the BIM is or contains a binding portion of the wild-type or unmodified ECD of TACI or a specific binding portion or fragment thereof that contains only the CRD2 of TACI and that binds to APRIL, BAFF and/or an APRIL/BAFF heterotrimer. In some embodiments, the BIM contains the sequence set forth in SEQ ID NO: 528, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:528 and that binds to APRIL, BAFF or an APRIL/BAFF heterotrimer, or (iii) is a fragment or portion of (i) or (ii) containing a portion of the CRD2, in which the portion binds to APRIL, BAFF or an APRI/BAFF heterotrimer. In some embodiments, the BIM comprises the sequence set forth in SEQ ID NO: 528. In some embodiments, the BIM is a TACI sequence that consists or consists essentially of the sequence set forth in SEQ ID NO: 528.

**[0392]** In some embodiments, the BIM is a variant TACI containing an ECD or specific binding portion or fragment thereof having a vTD containing one or more amino acid substitutions (mutations or replacements) relative to a reference TACI sequence. The reference TACI sequence can include any as described in Section II above. The one more amino acid substitutions can include any of the amino acid substitutions described in Section II above. For example, the BIM can be a variant TACI containing an ECD or specific binding fragment thereof having a vTD containing any of the amino acid substitutions set forth in Table 2. In some examples, the mutations are made in a reference TACI containing the sequence of amino acids set forth in SEQ ID NO: 709. In some examples, the mutations are made in a reference TACI that contains the CRD1 and CRD2 domain of TACI, for example as set forth in SEQ ID NO: 516. In some examples, the mutations are made in a reference TACI that is further truncated by

deletion of N-terminal and C-terminal amino acid residues to retain the CRD2, for example as set forth in SEQ ID NO: 528.

**[0393]** In some embodiments, the BIM comprises an extracellular domain (ECD) sequence containing a CRD1 and CRD2, such as a variant TACI polypeptide set forth in any one of SEQ ID NOS: 517-527, 536, 537, 682-701. In some embodiments, the BIM is a variant TACI polypeptide that comprises a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 517-527, 536, 537, 682-701, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) TACI. In some embodiments, the BIM is a variant TACI polypeptide that comprises a specific binding fragment of any one of SEQ ID NOS: 517-527, 536, 537, 682-701, in which the specific binding fragment binds BAFF, APRIL or a BAFF/APRIL heterotimer and contains a contiguous sequence therein that contains the amino acid modification(s), e.g. substitution (s) therein not present in the reference (e.g., unmodified or wild-type) TACI.

**[0394]** In some embodiments, the BIM is a variant TACI polypeptide that consists or consists essentially of a variant TACI extracellular domain (ECD) sequences set forth in any one of SEQ ID NOS: 517-527, 536, 537, 682-701. In some embodiments, the BIM is a variant TACI polypeptide that consists or consists essentially of a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 517-527, 536, 537, 682-701, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) TACI. In some embodiments, the BIM is a variant TACI polypeptide that consists or consists essentially of a specific binding fragment of any one of SEQ ID NOS: 517-527, 536, 537, 682-701, in which the specific binding fragment binds BAFF, APRIL or an APRIL/BAFF heterotrimer and contains a contiguous sequence therein that contains the amino acid modification(s), e.g. substitution (s) therein not present in the reference (e.g., unmodified or wild-type) TACI.

**[0395]** In some embodiments, the BIM is a variant TACI polypeptide that comprises an extracellular domain (ECD) sequences containing a CRD2 but lacking the CRD1 of a reference TACI polypeptide, such as a variant TACI polypeptide set forth in any one of SEQ ID NOS: 529-535, 538-550, 673-681, 769-794. In some embodiments, the BIM is a variant TACI polypeptide that comprises a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 529-535, 538-550, 673-681, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-

type) TACI. In some embodiments, the BIM is a variant TACI polypeptide that comprises a specific binding fragment of any one of SEQ ID NOS: 529-535, 538-550, 673-681, 769-794, in which the specific binding fragment binds BAFF, APRIL or a BAFF/APRIL heterotimer and contains a contiguous sequence therein that contains the amino acid modification(s), e.g. substitution (s) therein not present in the reference (e.g., unmodified or wild-type) TACI.

**[0396]** In some embodiments, the BIM is a variant TACI polypeptide that consists or consists essentially of the sequence set forth in any one of SEQ ID NOS: 529-535, 538-550, 673-681, 769-794. In some embodiments, the BIM is a variant TACI polypeptide that consists or consists essentially of a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 529-535, 538-550, 673-681, 769-794, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) TACI. In some embodiments, the BIM is a variant TACI polypeptide that consists or consists essentially of a specific binding fragment of any one of SEQ ID NOS: 529-535, 538-550, 673-681, 769-794, in which the specific binding fragment binds BAFF, APRIL or a BAFF/APRIL heterotrimer, and contains a contiguous sequence therein that contains the amino acid modification(s), e.g. substitution (s) therein not present in the reference (e.g., unmodified or wild-type) TACI.

**[0397]** In some embodiments, the BIM is a variant TACI polypeptide that comprises the sequence set forth in SEQ ID NO:535. In some embodiments, the BIM is a variant TACI polypeptide that consists essentially of the sequence set forth in SEQ ID NO:535. In some embodiments, the BIM is a variant TACI polypeptide that consists of the sequence set forth in SEQ ID NO:535.

**[0398]** In some embodiments, the BIM is a variant TACI polypeptide that comprises the sequence set forth in SEQ ID NO:541. In some embodiments, the BIM is a variant TACI polypeptide that consists essentially of the sequence set forth in SEQ ID NO:541. In some embodiments, the BIM is a variant TACI polypeptide that consists of the sequence set forth in SEQ ID NO:541.

**[0399]** In some embodiments, the BIM is a variant TACI polypeptide that comprises the sequence set forth in SEQ ID NO:542. In some embodiments, the BIM is a variant TACI polypeptide that consists essentially of the sequence set forth in SEQ ID NO:542. In some embodiments, the BIM is a variant TACI polypeptide consists of the sequence set forth in SEQ ID NO:542.

**[0400]** In some embodiments, the BIM is a variant TACI polypeptide that comprises the sequence set forth in SEQ ID NO:688. In some embodiments, the BIM is a variant TACI polypeptide that consists essentially of the sequence set forth in SEQ ID NO:688. In some embodiments, the BIM is a variant TACI polypeptide that consists of the sequence set forth in SEQ ID NO:688.

### *2. BCMA*

**[0401]** In some embodiments, the BIM is or contains a wild-type BCMA ECD or a specific binding portion or fragment thereof containing a TD (e.g. a CRD) that binds to APRIL, BAFF and/or an APRIL/BAFF heterotrimer. In some embodiments, the BIM is or contains a variant BCMA ECD or a specific binding portion or fragment thereof containing a TD (e.g. a CRD) that binds to APRIL, BAFF and/or an APRIL/BAFF heterotrimer. In some embodiments, the BIM is a BCMA polypeptide or variant thereof with any of the sequences set forth in Section II above (e.g. Table 1).

**[0402]** In some embodiments, the BIM is or contains a wild-type or unmodified ECD of BCMA or a specific binding portion or fragment thereof containing a TD (e.g. a CRD) that binds to APRIL, BAFF and/or an APRIL/BAFF heterotrimer. In some embodiments, the BIM contains the ECD sequence set forth in SEQ ID NO: 710, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:710 and that binds to APRIL, BAFF or an APRIL/BAFF heterotrimer, or (iii) is a fragment or portion of (i) or (ii) containing a CRD, in which the portion binds to APRIL, BAFF or an APRI/BAFF heterotrimer. In some embodiments, the BIM is a BCMA sequence that consists or consists essentially of the sequence set forth in SEQ ID NO: 710. In some embodiments, the BIM is a BCMA sequence containing residues 2-54 of SEQ ID NO:710 that lacks the N-terminal methionine as set forth in SEQ ID NO: 710. In some embodiments, the BIM is a BCMA sequence that consists or consists essentially of the sequence set forth as amino acids 2-54 of SEQ ID NO: 710.

**[0403]** In some embodiments, the BIM contains the sequence set forth in SEQ ID NO: 356, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:356 and that binds to APRIL, BAFF or an APRIL/BAFF heterotrimer, or (iii) is a fragment or portion of (i) or (ii) containing a portion of a CRD, in which the portion binds to APRIL, BAFF or an APRI/BAFF heterotrimer. In some embodiments, the BIM comprises the sequence set forth in SEQ ID NO: 356. In some embodiments, the BIM is a BCMA sequence that consists or consists essentially of the sequence set forth in SEQ ID NO: 356.

**[0404]** In some embodiments, the BIM is a variant BCMA containing an ECD or specific binding portion or fragment thereof having a vTD containing one or more amino acid substitutions (mutations or replacements) relative to a reference BCMA sequence. The reference BCMA sequence can include any as described in Section II above. The one more amino acid substitutions can include any of the amino acid substitutions described in Section II above. For example, the BIM can be a variant BCMA containing an ECD or specific binding fragment thereof having a vTD containing any of the amino acid substitutions set forth in Table 1. In some examples, the mutations are made in a reference BCMA containing the sequence of amino acids set forth in SEQ ID NO: 710. In some examples, the mutations

are made in a reference BCMA that contains the CRD domain of BCMA. In some examples, the mutations are made in a reference BCMA set forth in SEQ ID NO: 356.

**[0405]** In some embodiments, the BIM comprises a variant BCMA polypeptide set forth in any one of SEQ ID NOS: 357-435. In some embodiments, the BIM is a variant BCMA polypeptide that comprises a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 357-435, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) BCMA. In some embodiments, the BIM is a variant BCMA polypeptide that comprises a specific binding fragment of any one of SEQ ID NOS: 357-435, in which the specific binding fragment binds BAFF, APRIL or a BAFF/APRIL heterotimer and contains a contiguous sequence therein that contains the amino acid modification(s), e.g. substitution (s) therein not present in the reference (e.g., unmodified or wild-type) BCMA.

**[0406]** In some embodiments, the BIM is a variant BCMA polypeptide that consists or consists essentially of a variant BCMA extracellular domain (ECD) sequences set forth in any one of SEQ ID NOS: 357-435. In some embodiments, the BIM is a variant BCMA polypeptide that consists or consists essentially of a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 357-435, and retains the amino acid modification(s), e.g. substitution(s) therein not present in the reference (e.g., unmodified or wild-type) BCMA. In some embodiments, the BIM is a variant BCMA polypeptide that consists or consists essentially of a specific binding fragment of any one of SEQ ID NOS: 357-435, in which the specific binding fragment binds BAFF, APRIL or an APRIL/BAFF heterotrimer and contains a contiguous sequence therein that contains the amino acid modification(s), e.g. substitution (s) therein not present in the reference (e.g., unmodified or wild-type) BCMA.

**[0407]** In some embodiments, the BIM is a variant BCMA polypeptide that comprises the sequence set forth in SEQ ID NO:381. In some embodiments, the BIM is a variant BCMA polypeptide that consists essentially of the sequence set forth in SEQ ID NO:381. In some embodiments, the BIM is a variant BCMA polypeptide that consists of the sequence set forth in SEQ ID NO:381.

**[0408]** In some embodiments, the BIM is a variant BCMA polypeptide that comprises the sequence set forth in SEQ ID NO:405. In some embodiments, the BIM is a variant BCMA polypeptide that consists essentially of the sequence set forth in SEQ ID NO:405. In some embodiments, the BIM is a variant BCMA polypeptide that consists of the sequence set forth in SEQ ID NO:405.

**[0409]** In some embodiments, the BIM is a variant BCMA polypeptide that comprises the sequence set forth in SEQ ID NO:406. In some embodiments, the BIM is a variant BCMA polypeptide that

consists essentially of the sequence set forth in SEQ ID NO:406. In some embodiments, the BIM is a variant BCMA polypeptide that consists of the sequence set forth in SEQ ID NO:406.

**[0410]** In some embodiments, the BIM is a variant BCMA polypeptide that comprises the sequence set forth in SEQ ID NO:410. In some embodiments, the BIM is a variant BCMA polypeptide that consists essentially of the sequence set forth in SEQ ID NO:410. In some embodiments, the BIM is a variant BCMA polypeptide that consists of the sequence set forth in SEQ ID NO:410.

**[0411]** In some embodiments, the BIM is a variant BCMA polypeptide that comprises the sequence set forth in SEQ ID NO:411. In some embodiments, the BIM is a variant BCMA polypeptide that consists essentially of the sequence set forth in SEQ ID NO:411. In some embodiments, the BIM is a variant BCMA polypeptide that consists of the sequence set forth in SEQ ID NO:411.

### B. T CELL INHIBITORY MOLECULE (TIM)

**[0412]** In some embodiments, the provided immunomodulatory protein contains a TIM that binds to a T cell stimulatory receptor or to a ligand of a T cell stimulatory receptor. In some aspects, the T cell stimulatory receptor comprises a cytoplasmic region containing an immunoreceptor tyrosine-based activation motif (ITAM) or a cytoplasmic region that interacts with one or more adaptor protein involved in a signal transduction pathway in a cell to induce, mediate or potentiate activation of a T cell. In some embodiment, the adaptor protein contains a binding domain specific to a phosphotyrosine residue in a cytoplasmic region of stimulatory receptor. In some embodiments, the T cell stimulatory receptor includes a component of a TCR complex or is a co-receptor or costimulatory molecule that augments or enhances TCR signaling. In some embodiments, the T cell stimulatory receptor is a TCR, CD3, CD4, CD8, CD28, ICOS or CD2, including any mammalian orthologs thereof. In some embodiments, the T cell stimulatory receptor target is a human TCR, human CD3, human CD4, human CD8, human CD28, human ICOS or human CD2. In some embodiments, the T cell stimulatory receptor is expressed on a human T cell.

**[0413]** In some embodiments, the TIM binds directly to a T cell stimulatory receptor, such as directly to a component of a TCR complex or a co-receptor or costimulatory molecule that augments or enhances TCR signaling. In some embodiments, the TIM binds to a TCR, CD3, CD4, CD8, CD28, ICOS or CD2, including any mammalian orthologs thereof. In some embodiments, the TIM binds to a human TCR, human CD3, human CD4, human CD8, human CD28, human ICOS or human CD2.

**[0414]** In some cases, the TIM binds to a ligand of a T cell stimulatory receptor. In some embodiments, the TIM binds to a ligand of a component of a TCR complex or a ligand of a co-receptor or costimulatory molecule that augments or enhances TCR signaling. In some embodiments, the TIM binds to a ligand of a TCR, CD3, CD4, CD8, CD28, ICOS or CD2 molecule, including such molecules expressed on a T cell, e.g. a human T cell. In some embodiments, the TIM binds to a ligand of CD28,

such as a ligand of CD28 expressed on a T cell, e.g. a human T cell. In some embodiments, the ligand is a CD80 or a CD86, such as a human CD80 or human CD86. In some embodiments, the ligand is expressed on an APC.

**[0415]** In some embodiments, the TIM is an antibody or antigen-binding fragment that binds to a T cell stimulatory receptor or binds to a ligand of a T cell stimulatory receptor. In some embodiments, the TIM is an antibody or antigen-binding fragment that binds to a TCR, CD3, CD4, CD8, CD28, ICOS or CD2, including any mammalian orthologs thereof. In some embodiments, the antibody or antigen-binding fragment binds to a human TCR, human CD3, human CD4, human CD8, human CD28, human ICOS or human CD2, including such molecules expressed on a human T cell. In some embodiments, the antibody or antigen-binding fragment binds to CD80 or CD86. In some embodiments, the antibody or antigen-binding fragment binds to a human CD80 or human CD86, including such molecules expressed on a human APC.

**[0416]** In some embodiments, the TIM is or contains a binding partner of a T cell stimulatory receptor or a ligand of a T cell stimulatory receptor. Among molecules that can be used as a TIM are extracellular domains of IgSF protein member, particularly IgSF members that are T cell stimulatory receptors or their ligands.

**[0417]** In some aspects, the TIM is or contains an IgD of an IgSF family member that binds to an T cell stimulatory receptor, such as binds to TCR, CD3, CD4, CD8, CD28, ICOS or CD2, or is a specific fragment or vIgD thereof that binds to the T cell stimulatory receptor. In particular embodiments, the T cell stimulatory receptor is CD28 or ICOS and the TIM binds to CD28 or ICOS. Exemplary IgSF family members that are binding partners of or that bind to CD28 or ICOS include, for example, CD80, CD86 and ICOSL, such as human CD80, CD86 or ICOSL. In some examples, the TIM is or contains an IgD of a wild-type CD80, CD86 or ICOSL or is or contains a vIgD thereof, wherein the TIM specifically binds to CD28.

**[0418]** In other aspects, the TIM is or contains an IgD of an IgSF family member that binds to a ligand of a T cell stimulatory receptor, such as binds to CD80 or CD86, or is a specific fragment or vIgD thereof that binds to the ligand of the T cell stimulatory receptor. Exemplary IgSF family members that are binding partners of or that bind to CD80 or CD86 include, for example, CTLA-4, such as human CTLA-4. In some examples, the TIM is or contains an IgD of a wild-type CTLA-4 or is or contains a vIgD thereof, wherein the TIM specifically binds to CD80 or CD86. Exemplary sequences for inclusion as a TIM in the provided multi-domain immunomodulatory proteins include molecules described in International PCT published Appl. No. WO2019/074983.

**[0419]** In some embodiments, the multi-domain immunomodulatory protein provided herein are soluble proteins and/or do not contain a portion that includes a transmembrane domain. Those of skill will appreciate that cell surface proteins, including proteins of the IgSF, typically have an intracellular

domain, a transmembrane domain, and extracellular domain (ECD), and that a soluble form of such proteins can be made using the extracellular domain or an immunologically active subsequence thereof. Thus, in some embodiments, the TIM lacks a transmembrane domain or a portion of the transmembrane domain of an IgSF member. In some embodiments, the TIM lacks the intracellular (cytoplasmic) domain or a portion of the intracellular domain of an IgSF member. In some embodiments, the TIM only contains the ECD domain or a portion thereof containing an IgSF domain, such an IgV domain, or specific binding fragments thereof.

**[0420]** For example, in some aspects, the TIM is or contains an ECD of an IgSF receptor, or a specific binding portion or fragment thereof containing at least one IgD (e.g. IgV), that binds to a ligand of the T cell stimulatory receptor. For example, the TIM can contain an ECD of CTLA-4, or a specific binding portion or fragment of CTLA-4 containing at least one IgD (e.g. IgV), that binds to CD80 or CD86. In some embodiments, the TIM consists or consists essentially of an ECD of a an IgSF receptor, or a specific binding portion or fragment thereof containing at least one IgD (e.g. IgV), such as consists or consists essentially of the ECD of CTLA-4 or a specific binding portion or fragment of the ECD of CTLA-4 that contains an IgD (e.g. IgV). In some embodiments, the TIM is less than the full length sequence of the ECD of the receptor of the ligand of the T cell stimulatory receptor. In some embodiments, the TIM is or only contains one vIgD (e.g. IgV) or a specific binding fragment of the only one vIgD (e.g. IgV). In some embodiments, the TIM consists or consists essentially of an IgV of a receptor of the ligand of the T cell stimulatory receptor, such as consists or consists essentially of only the IgV of CTLA-4. In some embodiments, the sequence of the TIM containing an ECD or binding portion or fragment thereof containing a IgD (e.g.IgV) is a mammalian sequence that includes, but is not limited to, human, mouse, cynomolgus monkey, or rat. In some embodiments, the TIM sequence is human and/or binds a human protein.

**[0421]** In some aspects, the vIgD is an affinity-modified domain that exhibits increased binding activity, such as increased binding affinity, for the T cell stimulatory receptor or the ligand of the T cell stimulatory receptor compared to the binding activity of the unmodified or wild-type IgD for the same molecule. In some embodiment, the TIM contains a vIgD with one or more amino acid substitutions compared to an IgD of an IgSF member, e.g.CTLA-4 o, in which, the one or more amino acid substitutions confer or result in increased binding affinity to a cognate binding partner that is a T cell stimulatory receptor or a ligand of the T cell stimulatory receptor.

**[0422]** In some embodiments, the TIM is or contains a vIgD that contains one or more amino acids modifications, such as one or more substitutions (alternatively, “mutations” or “replacements”), deletions or additions, in an IgD relative to a wild-type or unmodified IgD of a binding partner of the T cell stimulatory receptor or a ligand of the T cell stimulatory receptor. In some aspects, the vIgD contains up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid modifications, such as

amino acid substitutions, deletions or additions in an IgD domain of an IgSF binding partner of a T cell stimulatory receptor or a ligand of a T cell stimulatory receptor. The modifications (e.g., substitutions) can be in the IgV domain or the IgC domain. In some embodiments, the vIgD has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modifications (e.g., substitutions) in the IgV domain or specific binding fragment thereof. In some embodiments, the vIgD has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modifications (e.g., substitutions) in the IgC domain or specific binding fragment thereof. In some embodiments, the vIgD has at least about 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the wild-type or unmodified IgD or specific binding fragment thereof.

**[0423]** Non-limiting examples of a TIM in the provided multidomain immunomodulatory proteins are described in the following subsections. Any of the described TIMs herein can be combined with a BIM as described in Section III.A.

### *1. Ligand-Binding TIM, e.g. CTLA-4*

**[0424]** Provided herein are immunomodulatory proteins containing a T cell molecule (TIM) that binds to a ligand of a T cell stimulatory receptor. In some aspects, the T cell stimulatory receptor is CD28, e.g. human CD28, and/or the ligand of the T cell stimulatory receptor is CD80 or CD86, e.g. human CD80 or human CD86. In some embodiments, the TIM of the immunomodulatory protein binds to the extracellular portion or ectodomain of CD80 or CD86. In some embodiments, the TIM binds to CD80 or CD86 on the surface of a cell, such as on the surface of an APC.

**[0425]** In some embodiments, the TIM is an antibody that binds CD80 or CD86 or is an antigen-binding antibody fragment thereof (e.g. Fab or scFv). In some embodiments, the antibody or antigen-binding antibody fragment binds human CD80 or human CD86. In some embodiments, the antibody is a single chain variable fragment (e.g. scFv) containing a VH and VL of an anti-CD80 antibody or antigen-binding fragment or an anti-CD86 antibody or antigen-binding fragment.

**[0426]** In some embodiments, the TIM is or contains an IgD (e.g. IgV) or a specific binding fragment thereof, such as an unmodified or wild-type IgD or a vIgD or a specific binding fragment thereof, of an IgSF family member that binds CD80 or CD86, such as human CD80 or human CD86. In some embodiments, the TIM is or contains one or more IgD that is an IgD, or a vIgD thereof, of a CTLA-4 polypeptide, such as a wild-type CTLA-4, e.g. a human CTLA-4. In some aspects, the TIM contains one or more IgD (e.g. IgV) that is an vIgD containing one or more amino acid modifications (e.g., substitutions, deletions or additions) compared to an IgD of a wild-type or unmodified CTLA-4, which, in some aspects, result in increased binding of the TIM to CD80 or CD86. Exemplary IgDs or vIgDs of CTLA-4 binding partners for inclusion as an TIM in the provided immunomodulatory proteins are described. In some embodiments, the TIM is or contains a vIgD polypeptide that exhibit increased

binding activity, such as binding affinity, for CD80 or CD86 compared to a corresponding wild-type or unmodified IgD. Exemplary IgDs or vIgDs of CTLA-4 for use as a TIM in the provided multi-domain immunomodulatory proteins include molecules described in International PCT published Appl. No. WO2019/074983.

**[0427]** CTLA-4 has been exploited as a therapeutic drug for treating autoimmune disease by attenuating T cell activation through modulation of CD80 and/or CD86 interactions. Specifically, abatacept and belatacept are FDA approved therapeutics for use in rheumatoid arthritis and transplant setting, respectively. Abatacept is wild-type CTLA-4 IgSF domain fused to an Fc portion of an antibody. The sequence of the CTLA-4 portion of abatacept is set forth in SEQ ID NO:1. Belatacept is a modified variant of CTLA-4 IgSF domain, containing a substitution of tyrosine for the alanine at position 31 and a glutamic acid for the leucine at position 106 (A31Y/L106E), corresponding to positions 31 and 106 of the wild-type reference CTLA-4 ECD sequence set forth in SEQ ID NO:1, to confer increased affinity toward CD80 and CD86 ligands (Kremer et al., *N Engl J Med*. 2003;349(20):1907-1915; Larsen et al, *Am J Transplant*. 2005;5(3):443-453). The sequence of the CTLA-4 portion of belatacept is set forth as SEQ ID NO: 672.

**[0428]** In some embodiments, the TIM is not the full length sequence of the CTLA-4. In some aspects, the TIM is a soluble polypeptide, is not membrane-expressed and/or lacks the transmembrane and/or cytoplasmic domain of CTLA-4. In some embodiments, the TIM only contains an extracellular domain (ECD) or a specific binding fragment thereof containing a IgD or vIgD, such as only contains an IgV domain or an IgC domain or specific binding fragment thereof, or combinations thereof.

**[0429]** In some embodiments, the TIM is or contains the ECD sequence set forth in SEQ ID NO:1 or is a specific binding fragment thereof. In some embodiments, the TIM is or contains the ECD sequence set forth in SEQ ID NO:2 or is a specific binding fragment thereof. In some embodiments, the TIM is or contains an IgD (e.g. IgV) sequence of CTLA-4, such as human CTLA-4. In some embodiments, the TIM is or contain an IgD (e.g. IgV) sequence set forth in SEQ ID NO:191, or is a specific binding fragment thereof.

KAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNEL TFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCP DSD (SEQ ID NO:1)

KAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNEL TFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCP DSDQ (SEQ ID NO:2)

HVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFL DDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYY (SEQ ID NO:191)

**[0430]** In some embodiments, the TIM contains the sequence set forth in SEQ ID NO: 1, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:1 and that binds to CD80 or CD86, or (iii) is a fragment or portion of (i) or (ii) containing an IgD (e.g. IgV) in which the portion binds to CD80 or CD86. In some embodiments, the TIM comprises the sequence set forth in SEQ ID NO: 1. In some embodiments, the TIM is a CTLA-4 sequence that consists or consists essentially of the sequence set forth in SEQ ID NO: 1.

**[0431]** In some embodiments, the TIM contains the sequence set forth in SEQ ID NO: 2, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:2 and that binds to CD80 or CD86, or (iii) is a fragment or portion of (i) or (ii) containing an IgD (e.g. IgV) in which the portion binds to CD80 or CD86. In some embodiments, the TIM comprises the sequence set forth in SEQ ID NO: 2. In some embodiments, the TIM is a CTLA-4 sequence that consists or consists essentially of the sequence set forth in SEQ ID NO: 2.

**[0432]** In some embodiments, the TIM contains the sequence set forth in SEQ ID NO: 191, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:191 and that binds to CD80 or CD86, or (iii) is a fragment or portion of (i) or (ii) containing an IgD (e.g. IgV) in which the portion binds to CD80 or CD86. In some embodiments, the TIM comprises the sequence set forth in SEQ ID NO: 191. In some embodiments, the TIM is a CTLA-4 sequence that consists or consists essentially of the sequence set forth in SEQ ID NO: 191.

**[0433]** In some embodiments, the immunomodulatory protein contains a TIM that is or contains a vIgD containing one or more amino acid modifications, e.g. substitutions, in an IgD of a wild-type or unmodified CTLA-4. In some embodiments, modifications provided herein can be in an TIM containing an unmodified IgD set forth in SEQ ID NO: 1, 2 or 191 or in a sequence that has 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 1, 2 or 191. In some embodiments, an TIM containing a vIgD of CTLA-4 has at least about 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the sequence set forth in any of SEQ ID NOs: 1, 2 or 191.

**[0434]** In some embodiments, the TIM is or contains a vIgD that is an affinity-modified IgSF domain that has an increased binding activity, such as binding affinity, for CD80 or CD86 relative to the binding activity of the wild-type or unmodified IgD for CD80 or CD86. In some embodiments, the

increase in binding activity, e.g. binding affinity, for CD80 or CD86 is increased at least about 5%, such as at least about 10%, 15%, 20%, 25%, 35%, 50%, 75%, 100%, 200% or more. In some embodiments, the increase in binding activity, e.g. binding affinity, is more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold 40-fold, or 50-fold. In such examples, the wild-type or unmodified IgD has the same sequence as the vIgD except that it does not contain the one or more amino acid modifications (e.g. substitutions).

**[0435]** In some embodiments, the equilibrium dissociation constant ($K_d$) of the TIM to CD80 or CD86 can be less than $1x10^{-5}$ M, $1x10^{-6}$ M, $1x10^{-7}$ M, $1x10^{-8}$ M, $1x10^{-9}$ M, $1x10^{-10}$ M or $1x10^{-11}$M, or $1x10^{-12}$ M or less. In some embodiments, the TIM binds to CD80 or CD86 with a $K_d$ of from or from about 100 pm to 5000 pm, 100 pm to 2000 pm, 100 pm to 1500 pm, 100 pm to 1000 pm, 100 pm to 800 pm, 100 pm to 500 pm, 100 pm to 400 pm, 400 pm to 4000 pm, 400 pm to 2000 pm, 400 pm to 1500 pm, 400 pm to 1000 pm, 400 pm to 800 pm, 400 pm to 500 pm, 500 pm to 5000 pm, 500 pm to 2000 pm, 500 pm to 1500 pm, 500 pm to 1000 pm, 500 pm to 800 pm, 800 pm to 5000 pm, 800 pm to 2000 pm, 800 pm to 1500 pm, 800 pm to 1000 pm, 1000 pm to 5000 pm, 1000 pm to 2000 pm, 1000 pm to 1500 pm, 1500 pm to 5000 pm, 1500 to 2000 pm to 2000 pm to 50000 pm. In some embodiments, the TIM binds to CD80 or CD86 with a $K_d$ of less than 200 pM, 300 pM, 400 pM, 500 pM. In some embodiments, the TIM binds to CD80 or CD86 with a $K_d$ of greater than or greater than about 500 pm but less than or less than about 2000 pm, such as from or from about 500 pm to 1500 pm, 500 pm to 1250 pm, 500 pm to 1000 pm, 500 pm to 750 pm, 750 pm to 1500 pm, 750 pm to 1250 pm, 750 pm to 1000 pm, 1000 pm to 2000 pm, 1000 pm to 1500 pm or 1500 pm to 2000 pm.

**[0436]** Unless stated otherwise, the amino acid modification(s) present in a vIgD of a CTLA-4 ECD or an IgD (e.g. IgV) thereof are designated by amino acid position number corresponding to the numbering of positions of the unmodified ECD sequence set forth in SEQ ID NO:1 or 2. It is within the level of a skilled artisan to identify the corresponding position of a modification, e.g. amino acid substitution, in an ECD or a portion thereof containing an IgSF domain (e.g. IgV) thereof, such as by alignment of a reference sequence with SEQ ID NOs: 1 or 2. In the listing of modifications throughout this disclosure, the amino acid position is indicated in the middle, with the corresponding unmodified (e.g. wild-type) amino acid listed before the number and the identified variant amino acid substitution listed after the number. If the modification is a deletion of the position a “del” is indicated and if the modification is an insertion at the position an “ins” is indicated. In some cases, an insertion is listed with the amino acid position indicated in the middle, with the corresponding unmodified (e.g. wild-type) amino acid listed before and after the number and the identified variant amino acid insertion listed after the unmodified (e.g. wild-type) amino acid.

**[0437]** In some embodiments, the TIM contains a vIgD that has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modifications, e.g. substitutions. The one or more amino

acid modifications, e.g. substitutions, can be in the ectodomain (extracellular domain) of the wild-type or unmodified CTLA-4. In some embodiments, the one or more amino acid modifications, e.g. substitutions, are in the ECD domain of CTLA-4 or a specific binding fragment thereof. In some embodiments, the one or more amino acid modifications, e.g. substitutions, are in the IgV domain of CTLA-4 or a specific binding fragment thereof. In some embodiments, the one or more amino acid modifications, e.g. substitutions, are in an IgC domain of CTLA-4 or a specific binding fragment thereof. In some embodiments, the one or more amino acid modifications, e.g. substitutions, are in the IgV domain of CTLA-4 or a specific binding fragment thereof and in an IgC domain or domains of CTLA-4 or a specific binding fragment thereof.

**[0438]** In some embodiments, the TIM is or contains a vIgD that has one or more amino modification, e.g. substitutions, in an unmodified IgD of CTLA-4 or specific binding fragment thereof corresponding to position(s) 6, 10, 12, 14, 15, 16, 18, 19, 20, 22, 24, 26, 27, 28, 29, 30, 31, 33, 35, 37, 38, 41, 42, 43, 45, 46, 47, 48, 53, 54, 55, 56, 58, 59, 61, 63, 64, 65, 67, 69, 71, 72, 73, 75, 76, 82, 85, 86, 87, 89, 91, 93, 95, 96, 97, 98, 99, 105, 106, 108, 110, 113, 115, 116, 117, I18, 119, 120, 121, 122, 124, 125 and/or 126 with reference to positions set forth in SEQ ID NO:1.

**[0439]** In some embodiments, the TIM contains a vIgD that has one or more amino acid modification, e.g. substitutions, in an unmodified CTLA-4 or specific binding fragment thereof corresponding to position(s) 12, 18, 26, 29, 31, 53, 56, 58, 63, 72, 98, 99, 105, 106, and/or 117 with reference to positions set forth in SEQ ID NO:1 or 2. In some embodiments, the TIM is or contains a vIgD of CTLA-4 that has one or more amino acid modifications selected from L12F, L12H, L12P, I18A, I18F, I18N, I18T, I18V, A26D, A26S, A26T, G29R, G29W, A31Y, T53S, M56K, M56L, M56R, M56T, M56V, N58D, N58S, L63H, L63P, S72G, S72T, L98Q, L98R, M99I, M99L, Y105F, Y105L, L106E, L106I, L106R, I117E, I117L, I117M, and/or I117T, or a conservative amino acid substitution thereof.

**[0440]** In some embodiments, the TIM is or contains a vIgD of CTLA-4 that has one or more amino acid modifications selected from A6T, V10A, L12F, L12H, L12I, L12P, S14N, S15P, R16C, R16G, R16H, I18A, I18F, I18N, I18T, I18V, A19V, S20N, V22A, V22I, E24Q, A26D, A26S, A26T, S27P, P28L, G29R, G29W, K30R, A31Y, E33M, E33V, R35K, T37S, V38I, Q41L, A42S, A42T, A42V, D43N, Q45H, V46E, T47A, E48R, T53S, Y54F, M55R, M55T, M55V, M56K, M56L, M56R, M56T, M56V, N58D, N58S, E59D, E59G, T61A, T61I, T61N, T61R, T61S, L63H, L63P, D64E, D64N, D64V, D65G, I67N, I67T, I67V, T69A, T69I, T69S, T71A, T71I, S72G, S72T, S73R, N75D, Q76R, Q82H, Q82R, R85G, A86T, M87A, M87K, M87T, M87V, T89A, T89M, T89S, L91R, I93L, I93V, K95R, V96I, E97Q, L98Q, L98R, M99I, M99L, Y105F, Y105L, L106E, L106I, L106N, L106R,L106V, I108F, I108V, N110K, N110S, N110Y, Q113H, Y115H, Y115N, V116A, I117E, I117K, I117L, I117M, I117T, P119H, E120D, P121S, C122P, D124P, D124I, S125I, S125P, D126P, and/or D126T , or a conservative amino acid substitution thereof. In some embodiments, the TIM is or contains a vIgD that has one or

more amino acid modification from L12F, L12H, L12I, L12P, I18A, I18F, I18N, I18T, I18V, A26D, A26S, A26T, G29R, G29W, E33M, E33V, T53S, M55R, M55T, M55V, M56K, M56L, M56R, M56T, M56V, N58D, N58S, L63H, L63P, S72G, S72T, M87A, M87K, M87T, M87V, L98Q, L98R, M99I, M99L, Y105F, Y105L, L106I, L106N, L106R, L106V, I117E, I117K, I117L, I117M, and/or I117T, or a conservative amino acid substitution thereof. In some embodiments, the TIM is or contains a vIgD that has one or more amino acid modifications selected from I12F, L12P, I18T, A26T, G29W, T53S, M55T, M56K, M56T, N58S, S72G, M99L, L63P, L98Q, Y105L, L106I, and/or I117L, or a conservative amino acid substitution thereof. In some embodiments, the TIM is or contains a vIgD has one or more amino acid modifications selected from L12P, I18T, A26T, G29W, A31Y, T53S, M55T, M56K, N58S, S72G, M99L, L63P, L98Q, Y105L, L106E, L106I, and/or I117L, or a conservative amino acid substitution thereof. In some embodiments, the TIM is or contains a vIgD that has one or more amino acid modifications selected from A26T, G29W, L63P, S72G, L98Q, M99L, Y105L and/or L106I, or a conservative amino acid substitution thereof.

**[0441]** In some embodiments, the TIM is or contains a vIgD that has two or more amino acid modifications selected from among A6T, V10A, L12F, L12H, L12I, L12P, S14N, S15P, R16C, R16G, R16H, I18A, I18F, I18N, I18T, I18V, A19V, S20N, V22A, V22I, E24Q, A26D, A26S, A26T, S27P, P28L, G29R, G29W, K30R, A31Y, E33M, E33V, R35K, T37S, V38I, Q41L, A42S, A42T, A42V, D43N, Q45H, V46E, T47A, E48R, T53S, Y54F, M55R, M55T, M55V, M56K, M56L, M56R, M56T, M56V, N58D, N58S, E59D, E59G, T61A, T61I, T61N, T61R, T61S, L63H, L63P, D64E, D64N, D64V, D65G, I67N, I67T, I67V, T69A, T69I, T69S, T71A, T71I, S72G, S72T, S73R, N75D, Q76R, Q82H, Q82R, R85G, A86T, M87A, M87K, M87T, M87V, T89A, T89M, T89S, L91R, I93L, I93V, K95R, V96I, E97Q, L98Q, L98R, M99I, M99L, Y105F, Y105L, L106E, L106I, L106N, L106R,L106V, I108F, I108V, N110K, N110S, N110Y, Q113H, Y115H, Y115N, V116A, I117E, I117K, I117L, I117M, I117T, P119H, E120D, P121S, C122P, D124P, D124I, S125I, S125P, D126P, and/or D126T.

**[0442]** In some embodiments, the TIM is or contain a vIgD of CTLA-4 that has an amino acid substitution in an unmodified or wild-type CTLA-4 polypeptide or specific binding fragment thereof corresponding to A26T, G29W, T53S, L63P, S72G, L98Q, M99L, Y105L and/or L106I. In some embodiments, the TIM is or contains a vIgD of CTLA-4 that contains the amino acid substitutions A26T/G29W, A26T/T53S, A26T/L63P, A26T/S72G, A26T/L98Q, A26T/M99L, A26T/Y105L, A26T/L106I, A26T/G29W, G29W/T53S, G29W/L63P, G29W/S72G, G29W/L98Q, G29W/M99L, G29W/Y105L, G29W/L106I, A26T/T53S, G29W/T53S, T53S/L63P, T53S/S72G, T53S/L98Q, T53S/M99L, T53S/Y105L, or T53S/L106I, A26T/L63P, G29W/L63P, T53S/L63P, L63P/S72G, L63P/L98Q, L63P/M99L, L63P/Y105L, or L63P/L106I, A26T/S72G, G29W/S72G, T53S/S72G, L63P/S72G, S72G/L98Q, S72G/M99L, S72G/Y105L or S72G/L106I, A26T/L98Q, G29W/L98Q, T53S/L98Q, L63P/L98Q, S72G/L98Q, L98Q/M99L, L98Q/Y105L or L98Q/L106I, A26T/M99L,

G29W/M99L, T53S/M99L, L63P/M99L, S72G/M99L, L98Q/M99L, M99L/Y105L, M99L/L106I, A26T/Y105L, G29W/Y105L, T53S/Y105L, L63P/Y105L, S72G/Y105L, L98Q/Y105L, M99L/Y105L, Y105L/L106I, A26T/L106I, G29W/L106I, T53S/L106IL63P/L106I, S72G/L106I, L98Q/L106I, M99L/L106I , Y105L/L106I. The variant CTLA-4 polypeptide can include further amino acid modifications (e.g. substitutions), such as any described herein, in accord with provided embodiments.

**[0443]** In some embodiments, the amino acid modification(s), e.g. substitutions(s) are A31Y/L106E, A6T/A26T/M55T/M99L/Y105L, V10A/G29W/T53S/M56K/L63P/L98Q/Y105L/P121S, V10A/L63P/D64V/S72G/L98Q/M99L/Y105L, V10A/L63P/L98Q/Y105L, L12F/R16H/G29W/M56T/L98Q/Y105L, L12F/A26T/L63P/L98Q/Y105L/L106R, L12F/K30R/S72G/Q82R/L98Q/M99L/Y105L, L12H/I18V/A42T/M55T/N58D/L98R/Y105L/L106I/P121S, L12H/E33M/L98Q/Y105L, L12H/M55T/E59D/L63P/M99L, L12H/L63P/S72G/L98Q/Y105L, L12I/M55T/M56V/I67T/M99L/L106R/I108F, L12P/R16H/A26T/T61S/L63P/M87V/L98Q/M99L/Y105L/L106I/I117L, L12P/I18T/A26T/M55T/T69S/S72G/M99L/Y105L, L12P/A26T, L12P/A26T/L63P, L12P/A26T/L63P/S72G/T89M/L98Q/M99L/Y105L, L12P/G29W/L63P/S72G/L98Q/Y105L, L12P/G29W/L63P/S72G/L98Q/Y105L/L106I, L12P/A26T/L63P/L98Q/M99L/Y105L, L12P/A26T/L63P/L98Q/Y105L, L12P/A26T/L63P/L98Q/Y105L/L106I, L12P/G29W/D43N/N58S/L63P/L98Q/M99L/Y105L, L12P/M56V/L63P/V96I/L98Q/M99L/Y105L/Y115H, L12P/L63P/S72G/L98Q/M99L/Y105L, L12P/L63P/S72G/L98Q/M99L/Y105L/L106N, L12P/L63P/S72G/L98Q/M99L/Y105L/L106N/I117L, S14N/R16C/I18T/M56K/T61A/L63P/A86T/M99L, S15P/I18V/M56T/L98Q/M99L/Y105L, R16C/G29W/E33V/M55T/L63P/L98Q/Y105L, I18A/L63P/S72G/L98Q/Y105L, I18F/L63P/L98Q/M99L/Y105L/P121S, I18N/A26T/L63H/T89A/L98Q/M99L/Y105L, I18N/L63P/S72T/M87T/L98Q/Y105L/N110S, I18T/A26S/M55T/M56V/L63P/S72G/L98Q/M99L/Y105L/I117K, I18T/A26T/L63P/S72G/L98Q/Y105L, I18T/A26T/L63P/Q82R/L98Q/Y105L, I18T/G29R/L63P/S72G/L98Q/M99L/Y105L, I18T/G29W/L63P/L98Q/Y105L, I18T/E48R/L63P/T69S/L98Q/Y105L/N110Y, I18T/T61R/L63P/S72G/L98Q/M99L/Y105L, I18T/L63P/S72G/M87K/L98Q/M99L/Y105L, I18T/L63P/S72G/L98Q/M99L/Y105L, I18T/L63P/S72G/L98Q/Y105L/I108V, I18V/A26T/L63P/D64E/L98Q/Y105L/L106R/N110K, I18V/G29W/L63P/S72G/L98Q/Y105L, A19V/G29W/R35K/L63P/L98Q/M99L/Y105L, S20N/A26T/L63P/L98Q/M99L/Y105L, V22A/L63P/L98Q/M99L/Y105L/P119H, V22I/L63P/L98Q/Y105L/I117M, E24Q/L63P/S72G/L98Q/M99L/Y105L, A26D/S72G/L98Q/M99L/Y105L, A26T/A42V/Q45H/I67N/M87K/E97Q/M99L,

A26T/V46E/L63P/D65G/L98Q, A26T/T47A/M56K/L63P/S72G/Q82R/L98Q/M99L/Y105L,
A26T/T53S/M56K/L63P/L98Q/Y105L, A26T/T53S/L63P/L98Q/Y105L/L106I/I117L,
A26T/Y54F/M56K/M99L/Y105L, A26T/M55R/L98Q/M99L/Y105L,
A26T/M55T/L63P/S72G/L98Q/M99L/Y105L, A26T/M55T/L63P/L98Q/M99L/Y105L,
A26T/L63P/D65G/L98Q/M99L/Y105L, A26T/L63P/M87V/N110K/I117E,
A26T/L63P/S72G/L98Q/M99L/Y105L, A26T/L63P/S72G/L98Q/Y105L/L106I/I117L,
A26T/L63P/L98Q/M99L/Y105L, A26T/I67N/S72G/L98Q/M99L/Y105L,
S27P/M56K/L63P/S72G/S73R/T89A/M99L/Y105L/I117M,
P28L/E33V/L63P/S72G/L98Q/M99L/Y105L, P28L/E33V/L63P/S72G/L98R/M99L/Y105L,
G29W/T53S/M56K/N58S/L63P/M87V/L98Q/Y105L,
G29W/T53S/M56K/N58S/L63P/M87V/L98Q/Y105L/I108V,
G29W/T53S/M56K/N58S/L63P/M87V/L98Q/Y105L/P121S,
G29W/T53S/M56K/T61N/L63P/L98Q/Y105L, G29W/T53S/M56K/L63P/Q82H/L98Q/M99I/Y105L,
G29W/T53S/M56K/L63P/L98Q/Y105L, G29W/T53S/L63P/S72G/L98Q/Y105L,
G29W/M55V/E59G/L63P/L98Q/Y105L, G29W/M56T/L63P/L98Q/Y105L/L106I/I117L,
G29W/N58D/I67V/L98Q/M99L/Y105L, G29W/N58S/L63P/D64N/L98Q/M99L/Y105L,
G29W/N58S/L63P/T69I/L98Q/M99L/Y105L, G29W/N58S/L63P/S72G/L98Q/Y105L,
G29W/N58S/L63P/S72G/L98Q/Y105L/L106I, G29W/N58S/L63P/S72G/L98Q/Y105L/L106V,
G29W/N58S/L63P/S72G/M87V/L98Q/Y105L, G29W/N58S/L63P/Q82R/L98Q/Y105L,
G29W/N58S/L63P/M87T/L98Q/M99L/Y105L, G29W/N58S/L63P/L98Q/Y105L,
G29W/E59G/L63P/L98Q/Y105L, G29W/T61I/L63P/S72G/L98Q/M99L/Y105L,
G29W/L63P/D65G/S72G/L98Q/Y105L, G29W/L63P/I67V/S72G/L98Q/Y105L,
G29W/L63P/S72G/L98Q/Y105L/L106I, G29W/L63P/S72G/L98Q/Y105L/L106I/I117L,
G29W/L63P/S72G/L98Q/Y105L/I117L,
G29W/L63P/S72G/L98Q/Y105L/P121S,G29W/L63P/L98Q/M99L/Y105L,
G29W/S72G/Q76R/L98Q/Y105L/L106I/Q113H,
G29W/M87K/T89S/L98Q/M99L/Y105L/I108V/I117L, G29W/M87K/I93V/L98Q/M99L/Y105L,
G29W/L98Q/M99L/Y105L, E33M/A42T/L98Q/Y105L, E33M/L63P/S72G/L98Q/Y105L,
E33M/L63P/S72G/L98Q/Y105L/I108F, E33M/L63P/S72G/L98Q/Y105L/I117L,
E33M/Q82H/L98Q/M99L/Y105L, E33V/A42S/M55T/L98Q/M99L/Y105L, T37S/M56V/L98Q/Y105L,
V38I/L63P/S72G/L98Q/M99L/Y105L, Q41L/Y54F/M56K/M99L/I108F, T53S/M56V/L98Q/Y105L,
M55T/L63P/T71I/M99L/Y105L, M55T/S72G/L98Q/M99L/Y105L, M55T/E97Q/M99L/Y105F,
M56K/L63P/N75D/V96I/M99L/Y105L/L106I, M56L/L63P/L98Q/Y105L/L106I/I117L,
M56R/L63P/L98Q/M99L/Y105L, M56T/L91R/L98Q/Y105L,
M56V/E59G/L63P/S72G/M87K/I93V/L98Q/M99L/Y105L/I117E,

T61A/L63P/S72G/L98Q/M99L/Y105L, L63P/T69A/L98Q/M99L/Y105L/L106R/V116A, L63P/S72G/M87A/L98Q/Y105L, L63P/S72G/I93L/L98Q/M99L/Y105L, L63P/S72G/L98Q/M99L/Y105L, L63P/S72G/L98Q/M99L/Y105L/L106I/I117L, L63P/S72G/L98Q/Y105L, L63P/S72G/L98Q/Y105L/L106I/I117L, L63P/S72G/Y105L, L63P/M87K/M99L/L106R, L63P/Q82H/L98Q/M99L/Y105L, L63P/K95R, L63P/L98Q, L63P/L98Q/M99L/Y105L, L63P/L98Q/M99L/Y105L/L106I, L63P/L98Q/M99L/Y105L/I108V, L63P/L98Q/M99L/Y105L/I117M, L63P/L98Q/Y105L, L63P/L98Q/V116A, L63P/L98R/N110K, L63P/M99L/Y105L/I108F, I67V/S72G/Q82H/T89A/L98Q/M99L/Y105L, S72G/R85G/L98Q/M99L/Y105L/L106I, S72G/L98Q/M99L/Y105L/I117T, L98Q/M99L/Y105L, L98Q/M99L/Y105L/L106I/I117T, L98Q/M99L/Y105L/L106I/Y115N, L98Q/Y105L, and L98R/N110K.

**[0444]** In some embodiments, the TIM is or contains a vIgD of CTLA-4 that additionally includes the amino acid modifications C122S with reference to positions set forth in SEQ ID NO:1 or 2.

**[0445]** In some embodiments, the TIM is or contains a vIgD of CTLA-4 that includes the amino acid modifications C122S with reference to positions set forth in SEQ ID NO:1 or 2. In some embodiments, the TIM has the sequence of amino acids set forth in SEQ ID NO: 668.

**[0446]** In some embodiments, the TIM is or contains an IgD (e.g. IgV) of wild-type CTLA-4 set forth in Table 3 or a vIgD thereof comprising any of the modifications (e.g. substitutions) listed in Table 3. Table 3 also provides exemplary sequences by reference to SEQ ID NO for the extracellular domain (ECD) or IgV domain of CTLA-4. As indicated, the exact locus or residues corresponding to a given domain can vary, such as depending on the methods used to identify or classify the domain. Also, in some cases, adjacent N- and/or C-terminal amino acids of a given domain (e.g. IgV) also can be included in a sequence of an TIM, such as to ensure proper folding of the domain when expressed. Thus, it is understood that the exemplification of the SEQ ID NOS in Table 3 is not to be construed as limiting. For example, the particular domain, such as the IgV domain, of a variant CTLA-4 polypeptide can be several amino acids longer or shorter, such as 1-10, e.g. 1, 2, 3, 4, 5, 6 or 7 amino acids longer or shorter, than the sequence of amino acids set forth in the respective SEQ ID NO.

**[0447]** In some embodiments, the TIM is or contains a variant CTLA-4 ECD set forth in any one of SEQ ID NOS: 3-190. In some embodiments, the TIM is or contains a sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any one of SEQ ID NOS: 3-190 and contains the amino acid modification(s), e.g. substitution(s) not present in the wild-type or unmodified CTLA-4, e.g. not present in SEQ ID NO:1 or 2. In some embodiments, the TIM is or contains a specific binding fragment of any of the ECD sequences set forth in any one of SEQ ID NOS: 3-190 and that contains the amino acid modification(s), e.g. substitution(s) not present in the wild-type or unmodified CTLA-4, e.g. not present in SEQ ID NO:1 or 2.

**[0448]** In some embodiments, the TIM is or contains a variant IgV sequence set forth in any one of SEQ ID NOS: 192-355. In some embodiments, the TIM is or contains a sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any of the IgV sequences set forth in any one of SEQ ID NOS: 192-355 and contains the amino acid modification(s), e.g., substitution(s), not present in the wild-type or unmodified CTLA-4, e.g. not present in SEQ ID NO:191. In some embodiments, the TIM is a specific binding fragment of any of the IgV sequences set forth in any one of SEQ ID NOS: 192-355 and that contains the amino acid modification(s), e.g. substitution(s) not present in the wild-type or unmodified CTLA-4, e.g. set forth in SEQ ID NO:191.

**[0449]** In some embodiments, the TIM is a variant CTLA-4 polypeptide that comprises the sequence set forth in SEQ ID NO:92. In some embodiments, the TIM is a variant CTLA-4 polypeptide that consists essentially of the sequence set forth in SEQ ID NO:92. In some embodiments, the TIM is a variant CTLA-4 polypeptide that consists of the sequence set forth in SEQ ID NO:92.

**[0450]** In some embodiments, the TIM is a variant CTLA-4 polypeptide that comprises the sequence set forth in SEQ ID NO:113. In some embodiments, the TIM is a variant CTLA-4 polypeptide that consists essentially of the sequence set forth in SEQ ID NO:113. In some embodiments, the TIM is a variant CTLA-4 polypeptide that consists of the sequence set forth in SEQ ID NO:113.

**[0451]** In some embodiments, the TIM is a variant CTLA-4 polypeptide that comprises the sequence set forth in SEQ ID NO:165. In some embodiments, the TIM is a variant CTLA-4 polypeptide that consists essentially of the sequence set forth in SEQ ID NO:165. In some embodiments, the TIM is a variant CTLA-4 polypeptide that consists of the sequence set forth in SEQ ID NO:165.

**[0452]** In some embodiments, the TIM is a variant CTLA-4 polypeptide that comprises the sequence set forth in SEQ ID NO:186. In some embodiments, the TIM is a variant CTLA-4 polypeptide that consists essentially of the sequence set forth in SEQ ID NO:186. In some embodiments, the TIM is a variant CTLA-4 polypeptide that consists of the sequence set forth in SEQ ID NO:186.

**TABLE 3: Exemplary variant CTLA-4 TIMs containing an IgD or vIgD**

| **Mutation(s)** | **ECD SEQ ID NO** | **IgV SEQ ID NO** |
|---|---|---|
| Wild-type | 1,2 | 191 |
| C122S | 668 | |
| L12P/A26T/L63P/L98Q/Y105L | 3 | 192 |
| L63P/L98R/N110K | 4 | 193 |
| L12P/A26T | 5 | 194 |
| L12P/A26T/L63P | 6 | 195 |

| TABLE 3: Exemplary variant CTLA-4 TIMs containing an IgD or vIgD | | |
|---|---|---|
| **Mutation(s)** | **ECD SEQ ID NO** | **IgV SEQ ID NO** |
| L63P/L98Q/Y105L | 7 | 196 |
| L98Q/Y105L | 8 | 197 |
| L63P | 9 | 198 |
| L98R/N110K | 10 | 199 |
| L12P/A26T/L63P/L98Q/M99L/Y105L | 11 | 200 |
| E33M/Q82H/L98Q/M99L/Y105L | 12 | 201 |
| L63P/S72G/L98Q/M99L/Y105L | 13 | 202 |
| S14N/R16C/I18T/M56K/T61A/L63P/A86T/M99L | 14 | 203 |
| S27P/M56K/L63P/S72G/S73R/T89A/M99L/Y105L/I117M | 15 | 204 |
| M56K/L63P/N75D/V96I/M99L/Y105L/L106I | 16 | 205 |
| L63P/S72G/Y105L | 17 | 206 |
| L63P/L98Q/M99L/Y105L/I117M | 18 | 207 |
| L63P/S72G/L98Q/M99L/Y105L/L106I/I117L | 19 | 202 |
| A26T/L63P/S72G/L98Q/Y105L/L106I/I117L | 20 | 208 |
| L63P/L98Q/V116A | 21 | 209 |
| G29W/L98Q/M99L/Y105L | 22 | 210 |
| T37S/M56V/L98Q/Y105L | 23 | 211 |
| A26T/Y54F/M56K/M99L/Y105L | 24 | 212 |
| L12P/I18T/A26T/M55T/T69S/S72G/M99L/Y105L | 25 | 213 |
| V22I/L63P/L98Q/Y105L/I117M | 26 | 214 |
| A26T/L63P/S72G/L98Q/M99L/Y105L | 27 | 215 |
| E33M/A42T/L98Q/Y105L | 28 | 216 |
| M55T/E97Q/M99L/Y105F | 29 | 217 |
| M55T/S72G/L98Q/M99L/Y105L | 30 | 218 |
| R16C/G29W/E33V/M55T/L63P/L98Q/Y105L | 31 | 219 |
| L12P/A26T/L63P/L98Q/Y105L/L106I | 32 | 192 |
| M56L/L63P/L98Q/Y105L/L106I/I117L | 33 | 220 |
| S15P/I18V/M56T/L98Q/M99L/Y105L | 34 | 221 |
| I18T/G29W/L63P/L98Q/Y105L | 35 | 222 |
| L63P/Q82H/L98Q/M99L/Y105L | 36 | 223 |
| L98Q/M99L/Y105L/L106I/I117T | 37 | 224 |
| L98Q/M99L/Y105L/L106I/Y115N | 38 | 224 |
| M55T/L63P/T71I/M99L/Y105L | 39 | 225 |
| A26T/T53S/M56K/L63P/L98Q/Y105L | 40 | 226 |
| I18T/A26T/L63P/Q82R/L98Q/Y105L | 41 | 227 |
| L12H/M55T/E59D/L63P/M99L | 42 | 228 |
| I18T/L63P/S72G/L98Q/Y105L/I108V | 43 | 229 |
| I18T/L63P/S72G/L98Q/M99L/Y105L | 44 | 230 |
| T61A/L63P/S72G/L98Q/M99L/Y105L | 45 | 231 |
| V38I/L63P/S72G/L98Q/M99L/Y105L | 46 | 232 |
| L63P/S72G/I93L/L98Q/M99L/Y105L | 47 | 233 |
| L12I/M55T/M56V/I67T/M99L/L106R/I108F | 48 | 234 |

| TABLE 3: Exemplary variant CTLA-4 TIMs containing an IgD or vIgD | | |
|---|---|---|
| **Mutation(s)** | **ECD SEQ ID NO** | **IgV SEQ ID NO** |
| I18N/A26T/L63H/T89A/L98Q/M99L/Y105L | 49 | 235 |
| I18T/E48R/L63P/T69S/L98Q/Y105L/N110Y | 50 | 236 |
| I18N/L63P/S72T/M87T/L98Q/Y105L/N110S | 51 | 237 |
| G29W/M56T/L63P/L98Q/Y105L/L106I/I117L | 52 | 238 |
| G29W/N58S/L63P/M87T/L98Q/M99L/Y105L | 53 | 239 |
| G29W/N58S/L63P/D64N/L98Q/M99L/Y105L | 54 | 240 |
| I18T/L63P/S72G/M87K/L98Q/M99L/Y105L | 55 | 241 |
| M56V | 56 | 242 |
| L63P/K95R | 57 | 243 |
| L63P/L98Q | 58 | 209 |
| L98Q/M99L/Y105L | 59 | 224 |
| L63P/M87K/M99L/L106R | 60 | 244 |
| L63P/M99L/Y105L/I108F | 61 | 245 |
| V10A/L63P/L98Q/Y105L | 62 | 246 |
| M56T/L91R/L98Q/Y105L | 63 | 247 |
| A26T/L63P/M87V/N110K/I117E | 64 | 248 |
| G29W/L63P/L98Q/M99L/Y105L | 65 | 249 |
| A26T/V46E/L63P/D65G/L98Q | 66 | 250 |
| G29W/N58S/L63P/L98Q/Y105L | 67 | 251 |
| G29W/E59G/L63P/L98Q/Y105L | 68 | 252 |
| L12H/L63P/S72G/L98Q/Y105L | 69 | 253 |
| A6T/A26T/M55T/M99L/Y105L | 70 | 254 |
| A26T/L63P/D65G/L98Q/M99L/Y105L | 71 | 255 |
| V10A/L63P/D64V/S72G/L98Q/M99L/Y105L | 72 | 256 |
| L12P/G29W/D43N/N58S/L63P/L98Q/M99L/Y105L | 73 | 257 |
| I18V/A26T/L63P/D64E/L98Q/Y105L/L106R/N110K | 74 | 258 |
| A19V/G29W/R35K/L63P/L98Q/M99L/Y105L | 75 | 259 |
| L12P/A26T/L63P/S72G/T89M/L98Q/M99L/Y105L | 76 | 260 |
| P28L/E33V/L63P/S72G/L98R/M99L/Y105L | 77 | 261 |
| E24Q/L63P/S72G/L98Q/M99L/Y105L | 78 | 262 |
| I18T/G29R/L63P/S72G/L98Q/M99L/Y105L | 79 | 263 |
| L63P/L98Q/M99L/Y105L | 80 | 207 |
| Q41L/Y54F/M56K/M99L/I108F | 81 | 264 |
| S72G/L98Q/M99L/Y105L/I117T | 82 | 265 |
| M56R/L63P/L98Q/M99L/Y105L | 83 | 266 |
| E33M/L63P/S72G/L98Q/Y105L | 84 | 267 |
| L63P/L98Q/M99L/Y105L/L106I | 85 | 207 |
| A26T/M55R/L98Q/M99L/Y105L | 86 | 268 |
| L63P/S72G/M87A/L98Q/Y105L | 87 | 269 |
| A26D/S72G/L98Q/M99L/Y105L | 88 | 270 |
| V22A/L63P/L98Q/M99L/Y105L/P119H | 89 | 271 |
| A26T/M55T/L63P/L98Q/M99L/Y105L | 90 | 272 |

| TABLE 3: Exemplary variant CTLA-4 TIMs containing an IgD or vIgD | | |
|---|---|---|
| **Mutation(s)** | **ECD SEQ ID NO** | **IgV SEQ ID NO** |
| E33V/A42S/M55T/L98Q/M99L/Y105L | 91 | 273 |
| G29W/N58S/L63P/Q82R/L98Q/Y105L | 92 | 274 |
| E33M/L63P/S72G/L98Q/Y105L/I117L | 93 | 267 |
| A26T/I67N/S72G/L98Q/M99L/Y105L | 94 | 275 |
| L12F/A26T/L63P/L98Q/Y105L/L106R | 95 | 276 |
| S20N/A26T/L63P/L98Q/M99L/Y105L | 96 | 277 |
| G29W/T61I/L63P/S72G/L98Q/M99L/Y105L | 97 | 278 |
| G29W/N58S/L63P/T69I/L98Q/M99L/Y105L | 98 | 279 |
| L12P/L63P/S72G/L98Q/M99L/Y105L/L106N | 99 | 280 |
| L63P/T69A/L98Q/M99L/Y105L/L106R/V116A | 100 | 281 |
| G29W/N58S/L63P/S72G/L98Q/Y105L | 101 | 282 |
| G29W/L63P/D65G/S72G/L98Q/Y105L | 102 | 283 |
| T53S/M56V/L98Q/Y105L | 103 | 284 |
| L63P/S72G/L98Q/Y105L | 104 | 285 |
| I18A/L63P/S72G/L98Q/Y105L | 105 | 286 |
| G29W/T53S/M56K/L63P/L98Q/Y105L | 106 | 287 |
| I18V/G29W/L63P/S72G/L98Q/Y105L | 107 | 288 |
| G29W/L63P/S72G/L98Q/Y105L/L106I | 108 | 289 |
| G29W/L63P/I67V/S72G/L98Q/Y105L | 109 | 290 |
| G29W/M55V/E59G/L63P/L98Q/Y105L | 110 | 291 |
| G29W/L63P/S72G/L98Q/Y105L/I117L | 111 | 289 |
| L63P/S72G/L98Q/Y105L/L106I/I117L | 112 | 285 |
| L12F/R16H/G29W/M56T/L98Q/Y105L | 113 | 292 |
| L12P/G29W/L63P/S72G/L98Q/Y105L | 114 | 293 |
| L12P/G29W/L63P/S72G/L98Q/Y105L/L106I | 115 | 293 |
| G29W/L63P/S72G/L98Q/Y105L/L106I/I117L | 116 | 289 |
| G29W/N58S/L63P/S72G/L98Q/Y105L/L106I | 117 | 283 |
| A26T/T53S/L63P/L98Q/Y105L/L106I/I117L | 118 | 294 |
| G29W/N58S/L63P/S72G/M87V/L98Q/Y105L | 119 | 295 |
| G29W/S72G/Q76R/L98Q/Y105L/L106I/Q113H | 120 | 296 |
| G29W/N58S/L63P/S72G/L98Q/Y105L/L106V | 121 | 283 |
| A26T/L63P/L98Q/M99L/Y105L | 122 | 297 |
| G29W/N58D/I67V/L98Q/M99L/Y105L | 123 | 298 |
| I67V/S72G/Q82H/T89A/L98Q/M99L/Y105L | 124 | 299 |
| S72G/R85G/L98Q/M99L/Y105L/L106I | 125 | 300 |
| A26T/T47A/M56K/L63P/S72G/Q82R/L98Q/M99L/Y105L | 126 | 301 |
| A26T/M55T/L63P/S72G/L98Q/M99L/Y105L | 127 | 302 |
| L12H/I18V/A42T/M55T/N58D/L98R/Y105L/L106I/P121S | 128 | 303 |
| I18T/A26T/L63P/S72G/L98Q/Y105L | 129 | 304 |
| L12F/K30R/S72G/Q82R/L98Q/M99L/Y105L | 130 | 305 |
| L12P/L63P/S72G/L98Q/M99L/Y105L/L106N/I117L | 131 | 316 |
| G29W/M87K/I93V/L98Q/M99L/Y105L | 132 | 306 |

| TABLE 3: Exemplary variant CTLA-4 TIMs containing an IgD or vIgD | | |
|---|---|---|
| **Mutation(s)** | **ECD SEQ ID NO** | **IgV SEQ ID NO** |
| P28L/E33V/L63P/S72G/L98Q/M99L/Y105L | 133 | 307 |
| G29W/T53S/M56K/L63P/Q82H/L98Q/M99I/Y105L | 134 | 308 |
| I18F/L63P/L98Q/M99L/Y105L/P121S | 135 | 309 |
| L63P/L98Q/M99L/Y105L/I108V | 136 | 207 |
| A26T/A42V/Q45H/I67N/M87K/E97Q/M99L | 137 | 310 |
| M56V/E59G/L63P/S72G/M87K/I93V/L98Q/M99L/Y105L/I117E | 138 | 311 |
| G29W/M87K/T89S/L98Q/M99L/Y105L/I108V/I117L | 139 | 278 |
| L12P/M56V/L63P/V96I/L98Q/M99L/Y105L/Y115H | 140 | 312 |
| G29W/T53S/M56K/T61N/L63P/L98Q/Y105L | 141 | 313 |
| I18T/A26S/M55T/M56V/L63P/S72G/L98Q/M99L/Y105L/I117K | 142 | 314 |
| I18T/T61R/L63P/S72G/L98Q/M99L/Y105L | 143 | 315 |
| L12P/L63P/S72G/L98Q/M99L/Y105L | 144 | 316 |
| E33M/L63P/S72G/L98Q/Y105L/I108F | 145 | 267 |
| L12P/R16H/A26T/T61S/L63P/M87V/L98Q/M99L/Y105L/L106I/I117L | 146 | 317 |
| G29W/T53S/M56K/N58S/L63P/M87V/L98Q/Y105L/P121S | 147 | 318 |
| G29W/L63P/S72G/L98Q/Y105L/P121S | 148 | 289 |
| G29W/T53S/M56K/N58S/L63P/M87V/L98Q/Y105L | 149 | 318 |
| G29W/T53S/M56K/N58S/L63P/M87V/L98Q/Y105L/I108V | 150 | 318 |
| G29W/T53S/L63P/S72G/L98Q/Y105L | 151 | 319 |
| V10A/G29W/T53S/M56K/L63P/L98Q/Y105L/P121S | 152 | 320 |
| A31Y/L106E | 153, | 321 |
| A31Y/L106E/C122S | 155, | 321 |
| T89A/L98Q/M99L/Y105L/L106I/Y115N/E120D/C122P/D124P/S125I/D126P | 157 | 322 |
| N58S/L63P/T71A/S72G/L98Q/M99L/Y105L/D124I/S125P/D126T | 158 | 323 |
| R16G/E33M/N58S/E59G/L63P/L98Q/Y105L/E120D/C122P/D124P/S125I/D126P | 159 | 324 |
| G29W/L63P/S72G/L98Q/Y105L/P121S/D126T | 160 | 325 |
| L12H/E33M/L98Q/Y105L | 161 | 326 |
| T53S/M56K/N58S/L63P/M87V/L98Q/Y105L | 162 | 327 |
| I18T/A26T/M55T/M56K/L63P/L98Q/M99L/Y105L | 163 | 328 |
| I18T/A26T/M56K/L63P/L98Q/Y105L | 164 | 329 |
| T53S/L63P/L98Q | 165 | 330 |
| T53S/L63P/Y105L | 166 | 331 |
| T53S/M56K/N58S/L63P/M87V/L98Q | 167 | 332 |
| T53S/M56K/N58S/L63P/M87V/Y105L | 168 | 333 |
| T53S/M56K/N58S/L63P/L98Q/Y105L | 169 | 334 |
| T53S/M56K/N58S/M87V/L98Q/Y105L | 170 | 335 |
| T53S/M56K/L63P/M87V/L98Q/Y105L | 171 | 336 |
| T53S/N58S/L63P/M87V/L98Q/Y105L | 172 | 337 |
| M56K/N58S/L63P/M87V/L98Q/Y105L | 173 | 338 |
| E33V/L98Q/Y105L | 174 | 339 |
| E33V/M99L/Y105L | 175 | 340 |
| E33V/L98Q/M99L | 176 | 341 |

| TABLE 3: Exemplary variant CTLA-4 TIMs containing an IgD or vIgD | | |
|---|---|---|
| **Mutation(s)** | **ECD SEQ ID NO** | **IgV SEQ ID NO** |
| E33V/M99L | 177 | 342 |
| L12F/R16H/G29W/M56T/L98Q | 178 | 343 |
| L12F/R16H/G29W/M56T/Y105L | 179 | 344 |
| L12F/R16H/G29W/L98Q/Y105L | 180 | 345 |
| L12F/R16H/M56T/L98Q/Y105L | 181 | 346 |
| G29W/M56T/L98Q/Y105L | 182 | 347 |
| L12F/G29W/L98Q/Y105L | 183 | 348 |
| L12F/L98Q/Y105L | 184 | 349 |
| R16H/L98Q/Y105L | 185 | 350 |
| G29W/L98Q/Y105L | 186 | 351 |
| M56T/L98Q/Y105L | 187 | 352 |
| L12F/R16H/G29W/M56T/S72G/L98Q/Y105L | 188 | 353 |
| G29W/M56T/S72G/L98Q/Y105L | 189 | 354 |
| I18T/T61R/L63P/S72G/L98Q/M99L/P102L/Y105L | 190 | 355 |

### C. FORMATS

**[0453]** The multi-domain immunomodulatory proteins containing one or more BIM and one or more TIM provided herein can be formatted in a variety of ways, including as a single chain polypeptide fusion or as a multimeric (e.g. dimeric, trimeric, tetrameric, or pentameric) molecules. The particular format is chosen such that the BIM of the immunomodulatory protein specifically binds to a ligand of a B cell stimulatory receptor and the TIM specifically binds to a T cell stimulatory receptor or a ligand of a T cell stimulatory receptor. In some aspects, the particular format is chosen to effect attenuation of an activity of the T cell stimulatory receptor and the B cell stimulatory receptor, such as to reduce or decrease an immune response mediated by T cells and B cells, respectively. In some embodiments, the modular format of the provided immunomodulatory proteins provides flexibility for engineering or generating immunomodulatory proteins for modulating activity at an immune synapse involving interactions between a B cell stimulatory receptor and a T cell stimulatory receptor and their ligands.

**[0454]** In some embodiments, the format of the multi-domain immunomodulatory protein is chosen to avoid crosslinking or engagement of the T cell stimulatory receptor. Thus, in some aspects, the provided immunomodulatory proteins do not exhibit multivalent binding to the T cell stimulatory receptor. For example, for the immunomodulatory proteins generated in which the TIM binds to a T cell stimulatory receptor, e.g. CD28, a relatively smaller molecular weight, monomeric and/or single chain polypeptide fusion of the immunomodulatory protein is contemplated.

**[0455]** In some embodiments, the provided multi-domain immunomodulatory proteins can include one or more BIM and one or more TIM. In some embodiments, an immunomodulatory protein can include one or more BIM described herein and any one or more TIM described herein. In some embodiments, the immunomodulatory protein comprises exactly 1, 2, 3, 4, 5 BIMs, which, in some aspects, are the same or are of identical sequence when a plurality are included. In some embodiments, each of a plurality of , e.g. 2, 3, 4, or 5, are linked directly or indirectly via a linker to another BIM. In some embodiments, the immunomodulatory proteins comprises exactly 1, 2, 3, 4, 5 TIMs, which, in some aspects, are the same or are of identical sequence when a plurality are included. In some embodiments, each of a plurality of TIMs, e.g. 2, 3, 4, or 5, are linked directly or indirectly via a linker to another TIM.

**[0456]** In some embodiments, the multi-domain immunomodulatory protein contains a polypeptide that includes at least one BIM and at least one TIM. In some aspects, at least one of the one or more BIM molecules are linked directly or indirectly via a linker to at least one of the one or more TIM. In some embodiments, the immunomodulatory protein includes a polypeptide containing a BIM linked directly or indirectly via a linker to a TIM, in either order. In some embodiments, at least one BIM is amino terminal to at least one TIM in the polypeptide. In some embodiments, at least one BIM is carboxy terminal to at least one TIM in the polypeptide.

**[0457]** In addition to single polypeptide chain embodiments, in some embodiments two, three, four, or more of a polypeptides containing one or more BIM and/or one or more BIM can be covalently or non-covalently attached to each other. In some embodiments, at least one polypeptide chain contains one or more BIM and at least one polypeptide chain contains one or more TIM. In some embodiments, each of at least two polypeptide chain contains at least one BIM and at least one TIM. Thus, monomeric, dimeric, and higher order (e.g., 3, 4, 5, or more) multimeric proteins are provided herein. For example, in some embodiments exactly two polypeptides, each containing one or more BIM and/or one or more TIM, can be covalently or non-covalently attached to each other to form a dimer. In some embodiments, the two polypeptides can be attached via a multimerization domain, in which, in some aspects, one or both of the BIM and TIM are linked directly or indirectly via a linker to the multimerization domain. In such embodiments, the multimerization domain can be the same or different. In some embodiments, the multimerization domain, such as an Fc region, facilitates attachment of two polypeptide chains via interchain cysteine disulfide bond. Compositions comprising two or more polypeptides can be of an identical sequence or substantially identical sequence of polypeptide (e.g., a homodimer) or of a non-identical sequence of polypeptides (e.g., a heterodimer).

**[0458]** In some embodiments, the multi-domain immunomodulatory protein can further include a tag or moiety.

**[0459]** Non-limiting examples of components for inclusion in provided formats are further described in the following subsections. Exemplary Fc-fusion formats of provided multi-domain immunomodulatory proteins are depicted in **FIG. 16**.

### *I. LINKERS*

**[0460]** For the multi-domain immunomodulatory proteins provided herein, linkers (interchangeably used with the term spacers) can be used to connect components of a polypeptide, such as any BIM and/or TIM provided herein. In some cases, a linker is a peptide or polypeptide sequence {e.g. a synthetic peptide or polypeptide sequence), or is a non-peptide linker able to connect two moieties. In some aspects, a linker is used or chosen to maintain the structural flexibility and other conformational characteristics of the individual residues or at the secondary, tertiary, or quaternary structural levels of domains of the polypeptide fusion protein, such as in order to maintain functional properties of the immunomodulatory protein. Linkers can also provide additional beneficial properties to the protein, such as increased protein expression in mammalian expression systems, improved biophysical properties such as stability and solubility, improved protein purification and detection and/or increased enzymatic activity. In some examples, two or more linkers can be linked in tandem.

**[0461]** In some aspects, the linkers can be peptide linker. In other aspects, the linker includes chemical linking agents and heterobifunctional linking agents. In some cases, the linker is not cleavable. In other cases, a linker can contain one or more protease-cleavable sites, which can be located within the sequence of the linker or flanking the linker at either end of the linker sequence.

**[0462]** When multiple linkers are present in the immunomodulatory protein between BIM, TIM or other moieties, each of the linkers can be the same or different. Generally, linkers or multiple linkers provide flexibility to the polypeptide molecule.

**[0463]** In some embodiments, one or more “peptide linkers” link the BIM, TIM, or other moieties of the immunomodulatory protein. In some embodiments, a peptide linker can be a single amino acid residue or greater in length. In some embodiments, the peptide linker has at least one amino acid residue but is no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues in length. In some embodiments, the linker is a flexible linker. Linking moieties are described, for example, in Huston et al. (1988) PNAS 85:5879-5883, Whitlow et al. (1993) Protein Engineering 6:989-995, and Newton et al, (1996) Biochemistry 35:545-553. Other suitable peptide linkers include any of those described in U.S. Patent Nos. 4,751,180 or 4,935,233.

**[0464]** In some examples, a peptide linker includes peptides (or polypeptides) {e.g., natural, or non-naturally occurring peptides) which includes an amino acid sequence that links or genetically fuses a first linear sequence of amino acids to a second linear sequence of amino acids to which it is not naturally linked or genetically fused in nature. For example, the peptide linker can include non-naturally occurring

polypeptides which are modified forms of naturally occurring polypeptides (e.g., that includes a mutation such as an addition, substitution or deletion). In another example, the peptide linker can include non-naturally occurring amino acids. In another example, the peptide linker can include naturally occurring amino acids occurring in a linear sequence that does not occur in nature. In still another example, the peptide linker can include a naturally occurring polypeptide sequence. Linking moieties can also include derivatives and analogs of the naturally occurring amino acids, as well as various non-naturally occurring amino acids (D- or L-), hydrophobic or non-hydrophobic, known in the art.

**[0465]** Exemplary peptide linkers are linkers with the formula $Ser(Gly_4Ser)_n$ (or $(Gly\text{-}Ser)_n$ residues with some Glu or Lys residues dispersed throughout to increase solubility, where n can be an integer from 1 to 20, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. Other exemplary linkers include peptide linkers with the formula $[(Gly)_x\text{-}Ser_y]_z$ where x is from 1 to 4, y is 0 or 1, and z is from 1 to 50. In other examples, the peptide linker includes the sequence $G_n$, where n can be an integer from 1 to 100. In another example, the sequence of the peptide linker can be $(GA)_n$ or $(GGS)_n$.

**[0466]** In some embodiments, the linker is (in one-letter amino acid code): GGGGS ("4GS"; SEQ ID NO: 593) or multimers of the 4GS linker, such as repeats of 2, 3, 4, or 5 4GS linkers. In some embodiments, the peptide linker is the peptide linker is $(GGGGS)_2$ (SEQ ID NO: 594), $(GGGGS)_3$ (SEQ ID NO: 595), $(GGGGS)_4$ (SEQ ID NO: 600) or $(GGGGS)_5$ (SEQ ID NO: 671). In some embodiments, the linker also can include a series of alanine residues alone or in addition to another peptide linker (such as a 4GS linker or multimer thereof). In some embodiments, the number of alanine residues in each series is: 2, 3, 4, 5, or 6 alanines. In some embodiments, the linker is a rigid linker. For example, the linker is an α-helical linker. In some embodiments, the linker is (in one-letter amino acid code): EAAAK (SEQ ID NO:711) or multimers of the EAAAK linker, such as repeats of 2, 3, 4, 5 or 6 EAAAK linkers, such as set forth in SEQ ID NO: 712 (2xEAAAK), SEQ ID NO: 713 (3xEAAAK), SEQ ID NO: 714 (4xEAAAK), SEQ ID NO: 715 (5xEAAAK), or SEQ ID NO: 665 (6xEAAAK). In some embodiments, the linker can further include amino acids introduced by cloning and/or from a restriction site, for example the linker can include the amino acids GS (in one-letter amino acid code) as introduced by use of the restriction site BAMHI. In some embodiments, the linker (in one-letter amino acid code) is GSGGGGS (SEQ ID NO: 590) or GGGGSSA (SEQ ID NO: 596). In some examples, the linker is a 2xGGGGS followed by three alanines (GGGGSGGGGSAAA; SEQ ID NO:721).

**[0467]** In some embodiments, a polynucleotide encoding a desired peptide linker can be inserted between, and in the same reading frame as a polynucleotide encoding any TIM, BIM or other moiety in the provided immunomodulatory protein and between another moiety, using any suitable conventional technique.

### *2. MUTIMERIZATION DOMAIN*

**[0468]** In some embodiments, the immunomodulatory protein containing one or more BIM(s) and/or TIM(s) is multimeric, such as dimeric, trimeric, tetrameric, or pentameric. For the dimeric format, the immunomodulatory protein comprises a first polypeptide and a second polypeptide. In some embodiments, the first and/or second polypeptide is or contains a BIM, TIM, or both. In aspects, the BIM and/or TIM is linked, directly or indirectly via a linker, to a multimerization domain. In some aspects, the mutlimerization domain increase half-life of the molecule. The linker can include any as described above.

**[0469]** In one example, the immunomodulatory protein provided herein is a dimer. In some cases, the immunomodulatory protein is a homodimer that contains a first and second polypeptide subunit that are the same, i.e. each has the same amino acid sequence containing the identical BIM(s) and TIM(s). The homodimer can be formed by transforming a nucleic acid molecule encoding the variant polypeptide into a cell, which, upon secretion, results in covalent or non-covalent interaction between residues of polypeptide subunits to mediate formation of the dimer.

**[0470]** In another example, the immunomodulatory protein is a heterodimer that contains a first and second polypeptide subunit that are different. In such an example, one or both of the first or second polypeptide subunit contains a sequence of amino acids of a BIM and TIM. In some cases, both the first and second polypeptide subunit can contain a sequence of amino acids of a BIM and a sequence of amino acids of a TIM. The heterodimer can be formed by transforming into a cell both a first nucleic acid molecule encoding a first polypeptide subunit and a second nucleic acid molecule encoding a second different polypeptide subunit. In some aspects, the heterodimer is produced upon expression and secretion from a cell as a result of covalent or non-covalent interaction between residues of the two polypeptide subunits to mediate formation of the dimer. In such processes, generally a mixture of dimeric molecules is formed, including homodimers and heterodimers. For the generation of heterodimers, additional steps for purification can be necessary. For example, the first and second polypeptide can be engineered to include a tag with metal chelates or other epitope, where the tags are different. The tagged domains can be used for rapid purification by metal-chelate chromatography, and/or by antibodies, to allow for detection by western blots, immunoprecipitation, or activity depletion/blocking in bioassays.

**[0471]** Interaction of two or more polypeptides of the immunomodulatory proteins can be facilitated by their linkage, either directly or indirectly, to any moiety or other polypeptide that are themselves able to interact to form a stable structure. For example, separate encoded polypeptide chains can be joined by multimerization, whereby multimerization of the polypeptides is mediated by a multimerization domain. Typically, the multimerization domain provides for the formation of a stable protein-protein interaction between a first polypeptide and a second polypeptide.

**[0472]** In some embodiments, the two or more individual polypeptides of the immunomodulatory proteins can be joined by multimerization, such as joined as dimeric, trimeric, tetrameric, or pentameric molecules. In some cases, the individual polypeptides are the same. For example, a trimeric molecule can be formed from three copies of the same individual polypeptide. In other examples, a tetrameric molecule is generated from four copies of the same individual polypeptides. In further examples, a pentameric molecule is generated from five copies of the same individual polypeptides. In some embodiments of the configurations, the individual polypeptides of an immunomodulatory proteins containing a BIM and/or TIM are fused to a multimerization domain. The multimerization domain may be one that facilities dimerization, trimerization, tetramerization, or pentamerization of the polypeptide chains.

**[0473]** In some embodiments, the immunomodulatory protein forms a multimer, e.g., a dimer. In some embodiments, the dimer is a homodimer in which the two polypeptides of the immunomodoulatory protein are the same. In some embodiments, the dimer is a heterodimer in which the two polypeptides of the immunomodoulatory protein are different.

**[0474]** In some embodiments, a multimerization domain includes any capable of forming a stable protein-protein interaction. The multimerization domains can interact via an immunoglobulin sequence (e.g. Fc domain; see e.g., International Patent Pub. Nos. WO 93/10151 and WO 2005/063816 US; U.S. Pub. No. 2006/0024298; U.S. Pat. No. 5,457,035); leucine zipper (e.g. from nuclear transforming proteins fos and jun or the proto-oncogene c-myc or from General Control of Nitrogen (GCN4)) (ee e.g., Busch and Sassone-Corsi (1990) Trends Genetics, 6:36-40; Gentz et al., (1989) Science, 243:1695-1699); a hydrophobic region; a hydrophilic region; or a free thiol which forms an intermolecular disulfide bond between the chimeric molecules of a homo- or heteromultimer. In addition, a multimerization domain can include an amino acid sequence comprising a protuberance complementary to an amino acid sequence comprising a hole, such as is described, for example, in U.S. Pat. No. 5,731,168; International Patent Pub. Nos. WO 98/50431 and WO 2005/063816; Ridgway et al. (1996) Protein Engineering, 9:617-621. Such a multimerization region can be engineered such that steric interactions not only promote stable interaction, but further promote the formation of heterodimers over homodimers from a mixture of chimeric monomers. Generally, protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g., tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are optionally created on the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine). Exemplary multimerization domains are described below.

**[0475]** The BIM and/or TIM can be joined anywhere, but typically via its N- or C-terminus, to the N- or C-terminus of a multimerization domain to form a chimeric polypeptide. The linkage can be direct or indirect via a linker. Also, the chimeric polypeptide can be a fusion protein or can be formed by

chemical linkage, such as through covalent or non-covalent interactions. For example, when preparing a chimeric polypeptide containing a multimerization domain, nucleic acid encoding all or part of a BIM and/or TIM can be operably linked to nucleic acid encoding the multimerization domain sequence, directly or indirectly or optionally via a linker domain. In some cases, the construct encodes a chimeric protein where the C-terminus of the BIM and/or TIM is joined to the N-terminus of the multimerization domain. In some instances, a construct can encode a chimeric protein where the N-terminus of the BIM and/or TIM is joined to the N- or C-terminus of the multimerization domain.

**[0476]** A polypeptide multimer contains two chimeric proteins created by linking, directly or indirectly, two of the same or different BIM and/or TIM directly or indirectly to a multimerization domain. In some examples, where the multimerization domain is a polypeptide, a gene fusion encoding the BIM and/or TIM and multimerization domain is inserted into an appropriate expression vector. The resulting chimeric or fusion protein can be expressed in host cells transformed with the recombinant expression vector, and allowed to assemble into multimers, where the multimerization domains interact to form multivalent polypeptides. Chemical linkage of multimerization domains to the BIM and/or TIM can be effected using heterobifunctional linkers.

**[0477]** The resulting chimeric polypeptides, such as fusion proteins, and multimers formed therefrom, can be purified by any suitable method such as, for example, by affinity chromatography over Protein A or Protein G columns. Where two nucleic acid molecules encoding different polypeptides are transformed into cells, formation of homo- and heterodimers will occur. Conditions for expression can be adjusted so that heterodimer formation is favored over homodimer formation.

**[0478]** In some embodiments, the immunomodulatory protein comprises a BIM and/or TIM attached to an immunoglobulin Fc (yielding an "immunomodulatory Fc fusion.") In some embodiments, the attachment of the BIM and/or TIM is at the N-terminus of the Fc. In some embodiments, the attachment of the BIM and/or TIM is at the C-terminus of the Fc. In some embodiments, two or more BIM and/or TIM (the same or different) are independently attached at the N-terminus and at the C-terminus. Thus, homo- or heteromultimeric polypeptides can be generated from co-expression of separate BIM and/or TIM containing polypeptides. The first and second polypeptides can be the same or different. In some embodiments, the first and/or second polypeptide each contains two or more BIM and/or TIM linked to the Fc sequence. In some embodiments, the first and/or second polypeptide each contains three TIMs and one BIM linked to the Fc sequence. Exemplary Fc fusion formats of provided multi-domain immunomodulatory proteins are depicted in **FIG. 16**.

**[0479]** In some embodiments, the Fc is murine or human Fc. In some embodiments, the Fc is a mammalian or human IgGl, lgG2, lgG3, or lgG4 Fc regions.

**[0480]** In some embodiments, the Fc is derived from IgG1, such as human IgG1. In some embodiments, the Fc is an IgG1 Fc set forth in SEQ ID NO: 586 having an allotype containing residues

Glu (E) and Met (M) at positions 356 and 358 by EU numbering. In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 586 or a sequence of amino acids that exhibits at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 586. In other embodiments, the Fc is IgG1 Fc that contains amino acids of the human G1m1 allotype, such as residues containing Asp (D) and Leu (L) at positions 356 and 358, e.g. as set forth in SEQ ID NO:597. Thus, in some cases, an Fc provided herein can contain amino acid substitutions E356D and M358L to reconstitute residues of allotype G1 m1. In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 597 or a sequence of amino acids that exhibits at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 597.

**[0481]** In some embodiments, the Fc region has the amino acid sequence set forth in SEQ ID NO:597.

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:597)

**[0482]** In some embodiments, the variant Fc comprises the sequence set forth in SEQ ID NO: 755. In some embodiments, the variant Fc comprises the sequence set forth in SEQ ID NO:756. In some embodiments, an Fc region used in a construct provided herein can further lack a C-terminal lysine residue.

**[0483]** In some embodiments, the Fc is derived from IgG2, such as human IgG2. In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 726 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 726. In some embodiments, the Fc region is an IgG2 Fc region that has the sequence set forth in SEQ ID NO: 726. In some embodiments, the Fc region is an IgG2 Fc region that has the sequence set forth in SEQ ID NO: 822.

**[0484]** In some embodiments, the Fc is derived from IgG4, such as human IgG4. In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 727 or a sequence of amino acids that exhibits at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 727. In some embodiments, the IgG4 Fc is a stabilized Fc in which the CH3 domain of human IgG4 is substituted with the CH3 domain of human IgG1 and which exhibits inhibited aggregate formation, an antibody in which the CH3 and CH2 domains of human IgG4 are substituted with the CH3 and CH2 domains of human IgG1, respectively, or an antibody in which arginine at position 409 indicated in the EU index proposed by Kabat et al. of human IgG4 is substituted with lysine and which exhibits inhibited aggregate formation (see e.g. U.S.

Patent No. 8,911,726. In some embodiments, the Fc is an IgG4 containing the S228P mutation, which has been shown to prevent recombination between a therapeutic antibody and an endogenous IgG4 by Fab-arm exchange (see e.g. Labrijin et al. (2009) Nat. Biotechnol., 27(8): 767-71.) In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 728 or a sequence of amino acids that exhibits at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 728. In some embodiments, the Fc region is an IgG4 Fc region set forth in SEQ ID NO:728. In some embodiments, the Fc region is an IgG4 Fc region set forth in SEQ ID NO:823.

**[0485]** In some embodiments, the Fc region contains one more modifications to alter (e.g. reduce) one or more of its normal functions. In general, the Fc region is responsible for effector functions, such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell cytotoxicity (ADCC), in addition to the antigen-binding capacity, which is the main function of immunoglobulins. Additionally, the FcRn sequence present in the Fc region plays the role of regulating the IgG level in serum by increasing the in vivo half-life by conjugation to an in vivo FcRn receptor. In some embodiments, such functions can be reduced or altered in an Fc for use with the provided Fc fusion proteins.

**[0486]** In some embodiments, one or more amino acid modifications may be introduced into the Fc region, thereby generating an Fc region variant. In some embodiments, the Fc region variant has decreased effector function. There are many examples of changes or mutations to Fc sequences that can alter effector function. For example, WO 00/42072, WO2006019447, WO2012125850, WO2015/107026, US2016/0017041 and Shields *et al. J Biol. Chem.* 9(2): 6591-6604 (2001) describe exemplary Fc variants with improved or diminished binding to FcRs. The contents of those publications are specifically incorporated herein by reference.

**[0487]** In some embodiments, the provided immunomodulatory proteins comprise an Fc region that exhibits reduced effector functions, which makes it a desirable candidate for applications in which the half-life of the immunomodulatory protein *in vivo* is important yet certain effector functions (such as CDC and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the immunomodulatory protein lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 2 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see, *e.g.* Hellstrom, I. *et al. Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I *et al.*, *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); U.S. Pat. No. 5,821,337 (see Bruggemann, M. *et al.*, *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively,

non-radioactive assay methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96™ non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al. Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the immunomodulatory protein n is unable to bind C1q and hence lacks CDC activity. See, *e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M. S. *et al.*, *Blood* 101:1045-1052 (2003); and Cragg, M. S. and M. J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, *e.g.*, Petkova, S. B. *et al.*, *Int'l. Immunol.* 18(12):1759-1769 (2006)).

**[0488]** Immunomodulatory protein with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 by EU numbering (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327 by EU numbering, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

**[0489]** In some aspects, a wild-type Fc is modified by one or more amino acid substitutions to reduce effector activity or to render the Fc inert for Fc effector function. Exemplary effectorless or inert mutations include those described herein. In some embodiments, the Fc region of immunomodulatory proteins has an Fc region in which any one or more of amino acids at positions 234, 235, 236, 237, 238, 239, 270, 297, 298, 325, and 329 (indicated by EU numbering) are substituted with different amino acids compared to the native Fc region. Such alterations of Fc region are not limited to the above-described alterations, and include, for example, alterations such as deglycosylated chains (N297A and N297Q), IgG1-N297G, IgG1-L234A/L235A, IgG1-L234A/L235E/G237A, IgG1-A325A/A330S/P331S, IgG1-C226S/C229S, IgG1-C226S/C229S/E233P/L234V/L235A, IgG1- E233P/L234V/L235A/G236del/ S267K, IgG1-L234F/L235E/P331S, IgG1-S267E/L328F, IgG2-V234A/G237A, IgG2-H268Q/V309L/A330S/A331S, IgG4-L235A/G237A/E318A, and IgG4-L236E described in Current Opinion in Biotechnology (2009) 20 (6), 685-691; alterations such as G236R/L328R, L235G/G236R, N325A/L328R, and N325LL328R described in WO 2008/092117; amino acid insertions at positions 233, 234, 235, and 237 (indicated by EU numbering); and alterations at the sites described in WO 2000/042072.Certain Fc variants with improved or diminished binding to FcRs are described. (See, *e.g.*, U.S. Pat. No. 6,737,056; WO 2004/056312, WO2006019447 and Shields *et al.*, *J. Biol. Chem*. 9(2): 6591-6604 (2001).)

**[0490]** In some embodiments, there is provided an immunomodulatory protein comprising a variant Fc region comprising one or more amino acid substitutions which increase half-life and/or improve binding to the neonatal Fc receptor (FcRn). Antibodies with increased half-lives and improved binding to FcRn are described in US2005/0014934A1 (Hinton *et al.*) or WO2015107026. Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434 by EU numbering, *e.g.*, substitution of Fc region residue 434 (U.S. Pat. No. 7,371,826).

**[0491]** In some embodiments, the Fc region of the immunomodulatory protein comprises one or more amino acid substitutions C220S, C226S and/or C229S by EU numbering. In some embodiments, the Fc region of the immunomodulatory protein comprises one or more amino acid substitutions R292C and V302C. *See* also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

**[0492]** In some embodiments, alterations are made in the Fc region that result in diminished C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie *et al.*, *J. Immunol*. 164: 4178-4184 (2000).

**[0493]** In some embodiments, there is provided an immunomodulatory protein comprising a variant Fc region comprising one or more amino acid modifications, wherein the variant Fc region is derived from a wild-type IgG1, such as a wild-type human IgG1. In some embodiments, the wild-type IgG1 Fc can be the Fc set forth in SEQ ID NO: 586 having an allotype containing residues Glu (E) and Met (M) at positions 356 and 358 by EU numbering. In some embodiments, the variant Fc region is derived from the amino acid sequence set forth in SEQ ID NO: 586. In other embodiments, the wild-type IgG1 Fc contains amino acids of the human G1m1 allotype, such as residues containing Asp (D) and Leu (L) at positions 356 and 358, e.g. as set forth in SEQ ID NO:597. Thus, in some cases, the variant Fc is derived from the amino acid sequence set forth in SEQ ID NO:597.

**[0494]** In some embodiments, the Fc region lacks the C-terminal lysine corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 586 or 597 (corresponding to K447del by EU numbering).

**[0495]** In some embodiments, the Fc contains at least one amino acid substitution that is N82G by numbering of SEQ ID NO: 586 (corresponding to N297G by EU numbering). In some embodiments, the Fc further contains at least one amino acid substitution that is R77C or V87C by numbering of SEQ ID NO: 586 (corresponding to R292C or V302C by EU numbering). In some embodiments, the variant Fc region further comprises a C5S amino acid modification by numbering of SEQ ID NO: 586 (corresponding to C220S by EU numbering). For example, in some embodiments, the variant Fc region comprises the following amino acid modifications: N297G and one or more of the following amino acid

modifications C220S, R292C or V302C by EU numbering (corresponding to N82G and one or more of the following amino acid modifications C5S, R77C or V87C with reference to SEQ ID NO:586), e.g., the Fc region comprises the sequence set forth in SEQ ID NO:598.

**[0496]** In some embodiments, the variant Fc contains the amino acid substitutions L234A/L235E/G237A, by EU numbering. In some embodiments, the variant Fc contains the amino acid substitutions A330S/P331S, by EU numbering. In some embodiments, the variant Fc contains the amino acid substitutions L234A/L235E/G237A/ A330S/P331S (Gross et al. (2001) Immunity 15:289). In some embodiments, the variant Fc comprises the sequence set forth in SEQ ID NO: 757. In some embodiments, the variant Fc comprises the sequence set forth in SEQ ID NO:758. In some embodiments, an Fc region used in a construct provided herein can further lack a C-terminal lysine residue.

**[0497]** In some embodiments, the variant Fc using in immunomodulatory protein constructs provided herein can include effectorless mutations L234A, L235E and G237A by EU numbering. In some embodiments, a wild-type Fc is further modified by the removal of one or more cysteine residue, such as by replacement of the cysteine residues to a serine residue at position 220 (C220S) by EU numbering. Exemplary inert Fc regions having reduced effector function are set forth in SEQ ID NO: 599 and SEQ ID NO:591, which are based on allotypes set forth in SEQ ID NO:586 or SEQ ID NO: 597, respectively. In some embodiments, an Fc region used in a construct provided herein can further lack a C-terminal lysine residue. In some embodiments, the variant Fc region comprises one or more of the amino acid modifications C220S, L234A, L235E or G237A, e.g. the Fc region comprises the sequence set forth in SEQ ID NO:589, 591, 599 or 724. In some embodiments, the variant Fc comprises has the sequence set forth in SEQ ID NO: 589. In some embodiments, the variant Fc comprises has the sequence set forth in SEQ ID NO: 591. In some embodiments, the variant Fc comprises has the sequence set forth in SEQ ID NO: 599. In some embodiments, the variant Fc comprises has the sequence set forth in SEQ ID NO: 724.

**[0498]** In some embodiments, the Fc region is a variant Fc that has the sequence set forth in SEQ ID NO:589.

**[0499]** EPKSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:589)

**[0500]** In some embodiments, the Fc region is a variant Fc that has the sequence set forth in SEQ ID NO: 824.

DKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:824)

**[0501]** In some embodiments, the variant Fc region comprises one or more of the amino acid modifications C220S, L235P, L234V, L235A, G236del or S267K, e.g. the Fc region comprises the sequence set forth in SEQ ID NO:722. In some embodiments, the Fc region lacks the C-terminal lysine corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 586 (corresponding to K447del by EU numbering).

**[0502]** In some embodiments, the variant Fc region comprises one or more of the amino acid modifications C220S, R292C, N297G, V302C. In some embodiments, the Fc region lacks the C-terminal lysine corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 586 (corresponding to K447del by EU numbering). An exemplary variant Fc region for use in the immunomodulatory protein constructs is set forth in SEQ ID NO: 723.

**[0503]** In some embodiments, the variant Fc region comprises one or more of the amino acid modifications C220S/E233P/L234V/L235A/G236del/S267K. In some embodiments, the Fc region lacks the C-terminal lysine corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 586 (corresponding to K447del by EU numbering). An exemplary variant Fc region for use in the immunomodulatory protein constructs is set forth in SEQ ID NO: 725.

**[0504]** In some embodiments, the immunomodulatory protein is a homodimer that contains a first immunomodulatory Fc fusion polypeptide and a second immunomodulatory Fc fusion polypeptide in which the first and second polypeptide are the same. In some embodiments, a first Fc polypeptide fusion contains an Fc region and one or more BIM and/or TIM and a second polypeptide fusion contains the same Fc region and the same one or more BIM and/or TIM. In such embodiments, the Fc region can be any as described above.

**[0505]** Examples of such Fc regions for inclusion in an immunomodulatory polypeptide are set forth in Table 4.

**Table 4: Exemplary Fc Regions, wild-type or variant (effectorless)**

| **Fc mutations (EU numbering)** | **356E/358M allotype SEQ ID NO** | **356D/358L allotype SEQ ID NO** |
|---|---|---|
| (wild-type) | 586 | 597 (with C220S, K447del |
| C220S, R292C, N297G, V302C | 598 | |

| | | |
|---|---|---|
| C220S, R292C, N297G, V302C, K447del | 726 | |
| C220S, L234A, L235E, G237A | 599 | 591 |
| C220S, L234A, L235E, G237A, K447del | 724 | 589 |
| L234A, L235E, G237A, K447del, with deletion of hinge | | 824 |
| C220S, L235P, L234V, L235A, G236del,S267K | 722 | |
| C220S/E233P/L234V/L235A/G236del/S267K/K447del | 725 | |
| L234A, L235E, G237A, A330S, P331S | | 758 |
| L234A, L235E, G237A, A330S, P331S, with deletion of hinge | | 757 |

**[0506]** In some embodiments, there is provided an immunomodulatory protein comprising at least one BIM (e.g. as described in Section III.A), at least one TIM (e.g. any set forth in Section III. B), and variant Fc region that exhibits reduced effector activity compared to a wild-type IgG1 set forth in SEQ ID NO:586 or 597. In some embodiments, the variant Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS:591, 598, 599, 722, 589, 723, 724, 725, 757, 758 or 824 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 591, 598, 599, 722, 589, 723, 724, 725, 757, 758 or 824. For example, provided herein is an immunomodulatory protein comprising at least one BIM (e.g. as described in Section III.A), at least one TIM (e.g. any set forth in Section III. B), and variant Fc region set forth in SEQ ID NO:589. In embodiments, when produced and expressed from a cells, the provided immunomodulatory protein is a homodimer containing two identical polypeptide chains.

**[0507]** In some embodiments, the immunomodulatory protein contains a first immunomodulatory Fc fusion polypeptide and a second immunomodulatory Fc fusion polypeptide in which the first and second polypeptide are different. In some embodiments, a first Fc polypeptide fusion contains an Fc region and one or more BIM and/or TIM and a second polypeptide fusion contains an Fc region and one or more BIM and/or TIM. In such embodiments, the Fc region can be a region that promotes or facilitates formation of heterodimers.

**[0508]** In some embodiments, the Fc domain of one or both of the first and second immunomodulatory Fc fusion polypeptides comprise a modification (e.g. substitution) such that the interface of the Fc molecule is modified to facilitate and/or promote heterodimerization. Methods to promote heterodimerization of Fc chains include mutagenesis of the Fc region, such as by including a set of “knob-into-hole” mutations or including mutations to effect electrostatic steering of the Fc to favor attractive interactions among different polypeptide chains. In some embodiments, the Fc region of the heterodimeric molecule additionally can contain one or more other Fc mutation, such as any described

above. In some embodiments, the heterodimer molecule contains an Fc region with a mutation that reduces effector function. In some embodiments, such Fc regions contain mutations C220S, L234A, L235E and/or G237A by EU numbering. In some embodiments, any of the above mutations in an Fc backbone can be made in an allotype containing residues Glu (E) and Met (M) at positions 356 and 358 by EU numbering. In other embodiments, any of the above mutations in an Fc backbone can be made in an allotype containing residue Asp (D) and Leu (L) at positions 356 and 358 by EU numbering.

**[0509]** In some embodiments, modifications include introduction of a protuberance (knob) into a first Fc polypeptide and a cavity (hole) into a second Fc polypeptide such that the protuberance is positionable in the cavity to promote complexing of the first and second Fc-containing polypeptides. Amino acids targeted for replacement and/or modification to create protuberances or cavities in a polypeptide are typically interface amino acids that interact or contact with one or more amino acids in the interface of a second polypeptide.

**[0510]** In some embodiments, a first polypeptide that is modified to contain protuberance (knob) amino acids include replacement of a native or original amino acid with an amino acid that has at least one side chain which projects from the interface of the first polypeptide and is therefore positionable in a compensatory cavity (hole) in an adjacent interface of a second polypeptide. Most often, the replacement amino acid is one which has a larger side chain volume than the original amino acid residue. One of skill in the art knows how to determine and/or assess the properties of amino acid residues to identify those that are ideal replacement amino acids to create a protuberance. In some embodiments, the replacement residues for the formation of a protuberance are naturally occurring amino acid residues and include, for example, arginine (R), phenylalanine (F), tyrosine (Y), or tryptophan (W). In some examples, the original residue identified for replacement is an amino acid residue that has a small side chain such as, for example, alanine, asparagine, aspartic acid, glycine, serine, threonine, or valine.

**[0511]** In some embodiments, a second polypeptide that is modified to contain a cavity (hole) is one that includes replacement of a native or original amino acid with an amino acid that has at least one side chain that is recessed from the interface of the second polypeptide and thus is able to accommodate a corresponding protuberance from the interface of a first polypeptide. Most often, the replacement amino acid is one which has a smaller side chain volume than the original amino acid residue. One of skill in the art knows how to determine and/or assess the properties of amino acid residues to identify those that are ideal replacement residues for the formation of a cavity. Generally, the replacement residues for the formation of a cavity are naturally occurring amino acids and include, for example, alanine (A), serine (S), threonine (T) and valine (V). In some examples, the original amino acid identified for replacement is an amino acid that has a large side chain such as, for example, tyrosine, arginine, phenylalanine, or tryptophan.

**[0512]** The CH3 interface of human IgG1, for example, involves sixteen residues on each domain located on four anti-parallel β-strands which buries 1090 Å2 from each surface (see e.g., Deisenhofer et al. (1981) Biochemistry, 20:2361-2370; Miller et al., (1990) J Mol. Biol., 216, 965-973; Ridgway et al., (1996) Prot. Engin., 9: 617-621; U.S. Pat. No. 5,731,168). Modifications of a CH3 domain to create protuberances or cavities are described, for example, in U.S. Pat. No. 5,731,168; International Patent Applications WO98/50431 and WO 2005/063816; and Ridgway et al., (1996) Prot. Engin., 9: 617-621. In some examples, modifications of a CH3 domain to create protuberances or cavities are typically targeted to residues located on the two central anti-parallel β-strands. The aim is to minimize the risk that the protuberances which are created can be accommodated by protruding into the surrounding solvent rather than being accommodated by a compensatory cavity in the partner CH3 domain.

**[0513]** In some embodiments, the heterodimeric molecule contains a T366W mutation in the CH3 domain of the "knobs chain" and T366S, L368A, Y407V mutations in the CH3 domain of the "hole chain". In some cases, an additional interchain disulfide bridge between the CH3 domains can also be used (Merchant, A. M., et al., Nature Biotech. 16 (1998) 677-681) e.g. by introducing a Y349C mutation into the CH3 domain of the "knobs" or "hole" chain and a E356C mutation or a S354C mutation into the CH3 domain of the other chain. In some embodiments, the heterodimeric molecule contains S354C, T366W mutations in one of the two CH3 domains and Y349C, T366S, L368A, Y407V mutations in the other of the two CH3 domains. For example, the knob Fc may contain the sequence set forth in SEQ ID NO: 669, containing S354C and T366W, and a hole Fc set forth in SEQ ID NO: 670, containing mutations Y349C, T366S, L368A and Y407V). In some embodiments, the heterodimeric molecule comprises E356C, T366W mutations in one of the two CH3 domains and Y349C, T366S, L368A, Y407V mutations in the other of the two CH3 domains. In some embodiments, the heterodimeric molecule comprises Y349C, T366W mutations in one of the two CH3 domains and E356C, T366S, L368A, Y407V mutations in the other of the two CH3 domains. In some embodiments, the heterodimeric molecule comprises Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains. Examples of other knobs-in-holes technologies are known in the art, e.g. as described by EP 1 870 459 A1.

**[0514]** In some embodiments, an Fc variant containing CH3 protuberance (knob) or cavity(hole) modifications can be joined to a multi-domain immunomodulatory polypeptide anywhere, but typically via its N- or C-terminus, to the N- or C-terminus of the one or more BIM or TIM, such as to form a fusion polypeptide. The linkage can be direct or indirect via a linker. Typically, a knob and hole molecule is generated by co-expression of a first stacked immunomodulatory polypeptide linked to an Fc variant containing CH3 protuberance modification(s) with a second stacked immunomodulatory polypeptide linked to an Fc variant containing CH3 cavity modification(s).

**[0515]** Exemplary sequences for knob and hole Fc polypeptides are set forth in SEQ ID NOs: 716, and 717, respectively. In some embodiments, the knob or hold Fc region lacks the C-terminal lysine corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 586 (corresponding to K447del by EU numbering). Exemplary sequences for knob and hole Fc polypeptides are set forth in SEQ ID NOs: 669, and 670, respectively.

**[0516]** In some embodiments, there is provided an immunomodulatory protein comprising a first polypeptide containing at least one BIM (e.g. as described in Section III.A) and/or at least one TIM (e.g. any set forth in Section III. B), and a first variant Fc set forth in SEQ ID NO:716; and a second polypeptide containing at least one BIM (e.g. as described in Section III.A) and/or at least one TIM (e.g. any set forth in Section III. B), and a second variant Fc set forth in SEQ ID NO:717. In some embodiments, there is provided an immunomodulatory protein comprising a first polypeptide containing at least one BIM (e.g. as described in Section III.A) and/or at least one TIM (e.g. any set forth in Section III. B), and a first variant Fc set forth in SEQ ID NO:669; and a second polypeptide containing at least one BIM (e.g. as described in Section III.A) and/or at least one TIM (e.g. any set forth in Section III. B), and a second variant Fc set forth in SEQ ID NO:670. .In embodiments, when produced and expressed from a cells, the provided immunomodulatory protein is a heterodimer containing two different polypeptide chains. In example, one of the polypeptides can express a TIM and one of the polypeptides can express a BIM.

**[0517]** In some embodiments, the Fc region of each polypeptide of a heterodimer includes a mutation to altered charge polarity across the Fc dimer interface such that coexpression of electrostatically matched Fc chains support favorable attractive interactions thereby promoting desired Fc heterodimer formation, whereas unfavorable repulsive charge interactions suppress unwanted Fc homodimer formation (Guneskaran et al. (2010) JBC, 285: 19637-19646). In some embodiments, at least one polypeptide containing an BIM and/or TIM is linked directly or indirectly to an Fc containing mutations to positively charged residues (e.g. E356K, E357K and/or D399K by EU numbering; designated K chain set forth), such as set forth in SEQ ID NO:729. In such embodiments, the other polypeptide of the heterodimer containing an BIM and/or TIM is linked directly or indirectly to an Fc containing mutations to negatively charged residues (e.g. K370D, K392D and K409D by EU numbering; designated D chain), such as set forth in SEQ ID NO:730. When co-expressed in a cell, association between the K and D chains is possible but the chains do not substantially self-associate due to charge repulsion.

**[0518]** In some embodiments, there is provided an immunomodulatory protein comprising a first polypeptide containing at least one BIM (e.g. as described in Section III.A) and/or at least one TIM (e.g. any set forth in Section III. B), and a first variant Fc set forth in SEQ ID NO:729; and a second polypeptide containing at least one BIM (e.g. as described in Section III.A) and/or at least one TIM (e.g.

any set forth in Section III. B), and a second variant Fc set forth in SEQ ID NO:730. In embodiments, when produced and expressed from a cells, the provided immunomodulatory protein is a heterodimer containing two different polypeptide chains. In example, one of the polypeptides can express a TIM and one of the polypeptides can express a BIM.

**[0519]** In some embodiments, individual polypeptide of a multi-domain polypeptide or individual polypeptides of a single-domain polypeptide are linked to a multimerization domain that forms an immunomodulatory protein is a trimer, tetramer or pentamer. In some embodiments, the individual polypeptides of such a molecule are the same. In some embodiments, such a multimerization domain is a cartilage oligomeric matrix protein (COMP) assembly domain, a vasodilator-stimulated phosphoprotein (VASP) tetramerization domain or a ZymoZipper (ZZ) 12.6 domain.

**[0520]** In some embodiments, the multimerization domain is a portion of the cartilage oligomeric matrix protein (COMP) assembly domain (Voulgaraki et al., Immunology (2005) 115(3):337-346. In some examples, the COMP is or contains an amino acid sequence as set forth in SEQ ID NO: 734 (e.g. amino acids 29-72 of the full length COMP, Uniprot accession number P49747) or a sequence that has 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 734.

**[0521]** In some embodiments, the multimerization domain is a vasodilator-stimulated phosphoprotein (VASP) tetramerization domain (Bachmann et al., J Biol Chem (1999) 274(33):23549-23557). In some embodiments, the VASP is or contains an amino acid sequence as set forth in SEQ ID NO: 735 (e.g. amino acids 343-375 of the full length VASP; Uniprot accession number P50552) or a sequence that has 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 735.

**[0522]** In some embodiments,the multimerization domain is a ZymoZipper (ZZ) 12.6 domain. In some embodiments, the ZZ domain is or contains an amino acid sequence as set forth in SEQ ID NO: 736 (See U.S. Patent No. 7,655,439) or a sequence that has 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 736.

**[0523]** In some configurations, a first and second polypeptide of a heterodimeric Fc fusion protein can be linked to a moiety for detection and/or purification. In some aspects, the first and second polypeptide are linked to different tags or moieties. In some aspects, the tag or moiety of the first and second polypeptide is independently selected from a poly-histidine tag (HHHHHH; SEQ ID NO: 702), a flag-tag (DYKDDDDK; SEQ ID NO: 588), a Myc-tag, or fluorescent protein-tags (e.g., EGFP, set forth in SEQ ID NOs:731, 732 or 733). In some examples, the first polypeptide containing an BIM and the second polypeptide containing an TIM each further contain a moiety for detection and/or purification, such as a poly-histidine tag (HHHHHH; SEQ ID NO: 702) and/or a flag-tag (DYKDDDDK; SEQ ID NO: 588).

**[0524]** In some embodiments, the BIM and/or TIM is directly linked to the Fc sequence. In some embodiments, the BIM and/or TIM is indirectly linked to the Fc sequence, such as via a linker. In some embodiments, one or more "peptide linkers" link the BIM and/or TIM and the Fc domain. In some embodiments, a peptide linker can be a single amino acid residue or greater in length. In some embodiments, the peptide linker has at least one amino acid residue but is no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues in length. Exemplary linkers are set forth in subsection "Linker."

**[0525]** In some embodiments, the linker is (in one-letter amino acid code): GGGGS ("4GS"; SEQ ID NO: 593) or multimers of the 4GS linker, such as repeats of 2, 3, 4, or 5 4GS linkers. In some embodiments, the peptide linker is the peptide linker is $(GGGGS)_2$ (SEQ ID NO: 594), $(GGGGS)_3$ (SEQ ID NO: 595), $(GGGGS)_4$ (SEQ ID NO: 600) or $(GGGGS)_5$ (SEQ ID NO: 671). In some embodiments, the linker also can include a series of alanine residues alone or in addition to another peptide linker (such as a 4GS linker or multimer thereof). In some embodiments, the linker (in one-letter amino acid code) is GSGGGGS (SEQ ID NO: 590) or GGGGSSA (SEQ ID NO: 596). In some examples, the linker is a 2xGGGGS followed by three alanines (GGGGSGGGGSAAA; SEQ ID NO:721).

**[0526]** Also provided are nucleic acid molecules encoding the immunomodulatory protein. In some embodiments, for production of immunomodulatory protein, a nucleic acid molecule encoding the immunomodulatory protein is inserted into an appropriate expression vector. The resulting immunomodulatory protein can be expressed in host cells transformed with the expression where assembly between Fc domains occurs by interchain disulfide bonds formed between the Fc moieties to yield dimeric, such as divalent, immunomodulatory proteins.

**[0527]** The resulting immunomodulatory protein containing an BIM, TIM, and Fc, can be easily purified by affinity chromatography over Protein A or Protein G columns. For the generation of heterodimers, additional steps for purification can be necessary. For example, where two nucleic acids encoding different immunomodulatory proteins are transformed into cells, the formation of heterodimers must be biochemically achieved since immunomodulatory protein carrying the Fc-domain will be expressed as disulfide-linked homodimers as well. Thus, homodimers can be reduced under conditions that favor the disruption of interchain disulfides, but do no effect intra-chain disulfides. In some cases, different immunomodulatory protein monomers are mixed in equimolar amounts and oxidized to form a mixture of homo- and heterodimers. The components of this mixture are separated by chromatographic techniques. Alternatively, the formation of this type of heterodimer can be biased by genetically engineering and expressing immunomodulatory proteins containing Fc fusion molecules that contain one or more BIM and/or TIM using knob-into-hole methods described below.

### *3. TAGS OR MOIETIES*

**[0528]** In some embodiments, the one or more polypeptides containing a BIM and/or TIM in the provided immunomodulatory proteins can further include a tag or moiety. In some embodiments, the further moiety is a protein, peptide, small molecule or nucleic acid. In some cases, the immunomodulatory protein is linked, directly or indirectly to more than one further moiety, such as 2, 3, 4, 5, or 6, further moieties.

**[0529]** In some embodiments, the moiety is a half-life extending molecule. Exemplary of such half-life extending molecules include, but are not limited to, albumin, an albumin-binding polypeptide, Pro/Ala/Ser (PAS), a C-terminal peptide (CTP) of the beta subunit of human chorionic gonadotropin, polyethylene glycol (PEG), long unstructured hydrophilic sequences of amino acids (XTEN), hydroxyethyl starch (HES), an albumin-binding small molecule, or a combination thereof.

**[0530]** In some embodiments, the immunomodulatory polypeptide comprising BIM and/or TIM can include conformationally disordered polypeptide sequences composed of the amino acids Pro, Ala, and Ser (See e.g., WO2008/155134, SEQ ID NO: 904). In some cases, the amino acid repeat is at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more amino acid residues, wherein each repeat comprises (an) Ala, Ser, and Pro residue(s). Thus, provided herein is an immunomodulatory protein is a PASylated protein wherein the BIM and/or TIM are linked, directly or indirectly via a linker, to Pro/Ala/Ser (PAS). In some embodiments, one or more additional linker structures may be used.

**[0531]** In some embodiments, the moiety facilitates detection or purification of the immunomodulatory protein. In some cases, the immunomodulatory protein, such as at least one of or each polypeptide of a multimer (e.g. dimer, trimer, tetramer, or pentamer) thereof, comprises a tag or moiety, e.g. affinity or purification tag, linked. In some aspects, such a tag or moiety can be linked directly or indirectly via a linker to the N- and/or c-terminus of the polypeptide. Various suitable polypeptide tags and/or fusion domains are known, and include but are not limited to, a poly-histidine (His) tag (SEQ ID NO:702), a FLAG-tag (SEQ ID NO:588), a Myc-tag, and fluorescent protein-tags (e.g., EGFP, set forth in SEQ ID NOs:734, 735, or 736. In some cases, the tag is a His tag containing at least six histidine residues (set forth in SEQ ID NO:702).

**[0532]** In some cases, the immunomodulatory protein comprising a BIM and TIM further comprises various combinations of moieties. For example, the immunomodulatory protein comprising BIM or TIM further comprises one or more polyhistidine-tag and FLAG tag. In some cases, the combination of moieties, such as two or more moieties, can be included on the same polypeptide. In some cases, the combination of moieties, such as two or more moieties, can be included on different polypeptide, such as in connection with embodiments relating to heterodimeric immunomodulatory polypeptides.

## IV. NUCLEIC ACIDS, VECTORS AND METHODS FOR PRODUCING THE POLYPEPTIDES OR CELLS

**[0533]** Provided herein are isolated or recombinant nucleic acids collectively referred to as "nucleic acids" which encode any of the immunomodulatory proteins provided herein. In some embodiments, nucleic acids provided herein, including all described below, are useful in recombinant production (e.g., expression) of immunomodulatory proteins provided herein. In some embodiments, nucleic acids provided herein, including all described below, are useful in expression of immunomodulatory proteins provided herein, such as BCMA fusion proteins or multi-domain immunomodulatory proteins provided herein. The nucleic acids provided herein can be in the form of RNA or in the form of DNA, and include mRNA, cRNA, recombinant or synthetic RNA and DNA, and cDNA. The nucleic acids provided herein are typically DNA molecules, and usually double-stranded DNA molecules. However, single-stranded DNA, single-stranded RNA, double-stranded RNA, and hybrid DNA/RNA nucleic acids or combinations thereof comprising any of the nucleotide sequences of the invention also are provided.

**[0534]** In some cases, a heterologous (non-native) signal peptide can be added to the nucleic acid encoding the immunomodulatory protein. This may be desired, for example, in the case of expression of BCMA fusion proteins or provided multi-domain immunomodulatory proteins, which do not contain an amino terminal signal sequence. In some embodiments, the signal peptide is a signal peptide from an immunoglobulin (such as IgG heavy chain or IgG-kappa light chain), a cytokine (such as interleukin-2 (IL-2), or CD33), a serum albumin protein (e.g. HSA or albumin), a human azurocidin preprotein signal sequence, a luciferase, a trypsinogen (e.g. chymotrypsinogen or trypsinogen) or other signal peptide able to efficiently express and, in some aspects, secret a protein from a cell. Exemplary signal peptides include any described in the Table 5.

**TABLE 5. Exemplary Signal Peptides**

| SEQ ID NO | Signal Peptide | Peptide Sequence |
|---|---|---|
| SEQ ID NO: 737 | HSA signal peptide | MKWVTFISLLFLFSSAYS |
| SEQ ID NO: 738 | Ig kappa light chain | MDMRAPAGIFGFLLVLFPGYRS |
| SEQ ID NO: 739 | human azurocidin preprotein signal sequence | MTRLTVLALLAGLLASSRA |
| SEQ ID NO: 740 | IgG heavy chain signal peptide | MELGLSWIFLLAILKGVQC |
| SEQ ID NO: 741 | IgG heavy chain signal peptide | MELGLRWVFLVAILEGVQC |
| SEQ ID NO: 742 | IgG heavy chain signal peptide | MKHLWFFLLLVAAPRWVLS |
| SEQ ID NO: 743 | IgG heavy chain signal peptide | MDWTWRILFLVAAATGAHS |
| SEQ ID NO: 744 | IgG heavy chain signal peptide | MDWTWRFLFVVAAATGVQS |
| SEQ ID NO: 745 | IgG heavy chain signal peptide | MEFGLSWLFLVAILKGVQC |
| SEQ ID NO: 746 | IgG heavy chain signal peptide | MEFGLSWVFLVALFRGVQC |
| SEQ ID NO: 747 | IgG heavy chain signal peptide | MDLLHKNMKHLWFFLLLVAAPR WVLS |
| SEQ ID NO: 748 | IgG Kappa light chain signal sequences: | MDMRVPAQLLGLLLLWLSGARC |

| | | |
|---|---|---|
| SEQ ID NO: 749 | IgG Kappa light chain signal sequences: | MKYLLPTAAAGLLLLAAQPAMA |
| SEQ ID NO: 750 | Gaussia luciferase | MGVKVLFALICIAVAEA |
| SEQ ID NO: 751 | Human albumin | MKWVTFISLLFLFSSAYS |
| SEQ ID NO: 752 | Human chymotrypsinogen | MAFLWLLSCWALLGTTFG |
| SEQ ID NO: 753 | Human interleukin-2 | MQLLSCIALILALV |
| SEQ ID NO: 754 | Human trypsinogen-2 | MNLLLILTFVAAAVA |

**[0535]** In some embodiments, the immunomodulatory protein comprises a signal peptide when expressed, and the signal peptide (or a portion thereof) is cleaved from the immunomodulatory protein upon secretion.

**[0536]** Also provided herein are recombinant expression vectors and recombinant host cells useful in producing the immunomodulatory proteins, such as BCMA fusion proteins or multi-domain immunomodulatory proteins provided herein.

**[0537]** In any of the above provided embodiments, the nucleic acids encoding the immunomodulatory polypeptides provided herein can be introduced into cells using recombinant DNA and cloning techniques. To do so, a recombinant DNA molecule encoding an immunomodulatory polypeptide is prepared. Methods of preparing such DNA molecules are well known in the art. For instance, sequences coding for the peptides could be excised from DNA using suitable restriction enzymes. Alternatively, the DNA molecule could be synthesized using chemical synthesis techniques, such as the phosphoramidite method. Also, a combination of these techniques could be used. In some instances, a recombinant or synthetic nucleic acid may be generated through polymerase chain reaction (PCR). A DNA insert encoding an immunomodulatory protein can be cloned into an appropriate transduction/transfection vector as is known to those of skill in the art. Also provided are expression vectors containing the nucleic acid molecules.

**[0538]** In some embodiments, the expression vectors are capable of expressing the immunomodulatory proteins in an appropriate cell under conditions suited to expression of the protein. In some aspects, nucleic acid molecule or an expression vector comprises the DNA molecule that encodes the immunomodulatory protein operatively linked to appropriate expression control sequences. Methods of effecting this operative linking, either before or after the DNA molecule is inserted into the vector, are well known. Expression control sequences include promoters, activators, enhancers, operators, ribosomal binding sites, start signals, stop signals, cap signals, polyadenylation signals, and other signals involved with the control of transcription or translation.

**[0539]** In some embodiments, expression of the immunomodulatory protein is controlled by a promoter or enhancer to control or regulate expression. The promoter is operably linked to the portion of the nucleic acid molecule encoding the variant polypeptide or immunomodulatory protein.

**[0540]** The resulting recombinant expression vector having the DNA molecule thereon is used to transform an appropriate host. This transformation can be performed using methods well known in the art. In some embodiments, a nucleic acid provided herein further comprises nucleotide sequence that encodes a secretory or signal peptide operably linked to the nucleic acid encoding an immunomodulatory polypeptide such that a resultant soluble immunomodulatory polypeptide is recovered from the culture medium, host cell, or host cell periplasm. In other embodiments, the appropriate expression control signals are chosen to allow for membrane expression of an immunomodulatory polypeptide. Furthermore, commercially available kits as well as contract manufacturing companies can also be utilized to make engineered cells or recombinant host cells provided herein.

**[0541]** In some embodiments, the resulting expression vector having the DNA molecule thereon is used to transform, such as transduce, an appropriate cell. The introduction can be performed using methods well known in the art. Exemplary methods include those for transfer of nucleic acids encoding the receptors, including via viral, e.g., retroviral or lentiviral, transduction, transposons, and electroporation. In some embodiments, the expression vector is a viral vector. In some embodiments, the nucleic acid is transferred into cells by lentiviral or retroviral transduction methods.

**[0542]** Any of a large number of publicly available and well-known mammalian host cells, including mammalian T-cells or APCs, can be used in the preparing the polypeptides or engineered cells. The selection of a cell is dependent upon a number of factors recognized by the art. These include, for example, compatibility with the chosen expression vector, toxicity of the peptides encoded by the DNA molecule, rate of transformation, ease of recovery of the peptides, expression characteristics, bio-safety and costs. A balance of these factors must be struck with the understanding that not all cells can be equally effective for the expression of a particular DNA sequence.

**[0543]** In some embodiments, the host cell is a mammalian cell. 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 (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).

**[0544]** In some embodiments, the host cells can be a variety of eukaryotic cells, such as in yeast cells, or with mammalian cells such as Chinese hamster ovary (CHO) or HEK293 cells. In some embodiments, the host cell is a suspension cell and the polypeptide is engineered or produced in cultured suspension, such as in cultured suspension CHO cells, e.g. CHO-S cells. In some examples, the cell line is a CHO cell line that is deficient in DHFR (DHFR-), such as DG44 and DUXB11. In some

embodiments, the cell is deficient in glutamine synthase (GS), e.g. CHO-S cells, CHOK1 SV cells, and CHOZN((R)) GS-/- cells. In some embodiments, the CHO cells, such as suspension CHO cells, may be CHO-S-2H2 cells, CHO-S-clone 14 cells, or ExpiCHO-S cells.

**[0545]** In some embodiments, host cells can also be prokaryotic cells, such as with E. coli. The transformed recombinant host is cultured under polypeptide expressing conditions, and then purified to obtain a soluble protein. Recombinant host cells can be cultured under conventional fermentation conditions so that the desired polypeptides are expressed. Such fermentation conditions are well known in the art. Finally, the polypeptides provided herein can be recovered and purified from recombinant cell cultures by any of a number of methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, and affinity chromatography. Protein refolding steps can be used, as desired, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed in the final purification steps.

**[0546]** In some embodiments, the recombinant vector is a viral vector. Exemplary recombinant viral vectors include a lentiviral vector genome, poxvirus vector genome, vaccinia virus vector genome, adenovirus vector genome, adenovirus-associated virus vector genome, herpes virus vector genome, and alpha virus vector genome. Viral vectors can be live, attenuated, replication conditional or replication deficient, non-pathogenic (defective), replication competent viral vector, and/or is modified to express a heterologous gene product, e.g., the variant immunomodulatory polypeptides provided herein. Vectors for generation of viruses also can be modified to alter attenuation of the virus, which includes any method of increasing or decreasing the transcriptional or translational load.

**[0547]** Exemplary viral vectors that can be used include modified vaccinia virus vectors (see, e.g., Guerra et al., J. Virol. 80:985-98 (2006); Tartaglia et al., AIDS Research and Human Retroviruses 8: 1445-47 (1992); Gheradi et al., J. Gen. Virol. 86:2925-36 (2005); Mayr et al., Infection 3:6-14 (1975); Hu et al., J. Virol. 75: 10300-308 (2001); U.S. Patent Nos. 5,698,530, 6,998,252, 5,443,964, 7,247,615 and 7,368,116); adenovirus vector or adenovirus-associated virus vectors (see., e.g., Molin et al., J. Virol. 72:8358-61 (1998); Narumi et al., Am J. Respir. Cell Mol. Biol. 19:936-41 (1998); Mercier et al., Proc. Natl. Acad. Sci. USA 101:6188-93 (2004); U.S. Patent Nos. 6,143,290; 6,596,535; 6,855,317; 6,936,257; 7,125,717; 7,378,087; 7,550,296); retroviral vectors including those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), ecotropic retroviruses, simian immunodeficiency virus (SIV), human immunodeficiency virus (HIV), and combinations (see, e.g., Buchscher et al., J. Virol. 66:2731-39 (1992); Johann et al., J. Virol. 66: 1635-40 (1992); Sommerfelt et al., Virology 176:58-59 (1990); Wilson et al., J. Virol. 63:2374-78 (1989); Miller et al., J. Virol. 65:2220-24 (1991); Miller et al., Mol. Cell Biol. 10:4239 (1990); Kolberg, NIH Res. 4:43 1992; Cornetta et al., Hum. Gene Ther. 2:215 (1991)); lentiviral vectors including those based upon Human Immunodeficiency Virus (HIV-1), HIV-2,

feline immunodeficiency virus (FIV), equine infectious anemia virus, Simian Immunodeficiency Virus (SIV), and maedi/visna virus (see, e.g., Pfeifer et al., Annu. Rev. Genomics Hum. Genet. 2: 177-211 (2001); Zufferey et al., J. Virol. 72: 9873, 1998; Miyoshi et al., J. Virol. 72:8150, 1998; Philpott and Thrasher, Human Gene Therapy 18:483, 2007; Engelman et al., J. Virol. 69: 2729, 1995; Nightingale et al., Mol. Therapy, 13: 1121, 2006; Brown et al., J. Virol. 73:9011 (1999); WO 2009/076524; WO 2012/141984; WO 2016/011083; McWilliams et al., J. Virol. 77: 11150, 2003; Powell et al., J. Virol. 70:5288, 1996) or any, variants thereof, and/or vectors that can be used to generate any of the viruses described above. In some embodiments, the recombinant vector can include regulatory sequences, such as promoter or enhancer sequences, that can regulate the expression of the viral genome, such as in the case for RNA viruses, in the packaging cell line (see, e.g., U.S. Patent Nos.5,385,839 and 5,168,062).

**[0548]** In some aspects, nucleic acids or an expression vector comprises a nucleic acid sequence that encodes the immunomodulatory protein operatively linked to appropriate expression control sequences. Methods of effecting this operative linking, either before or after the nucleic acid sequence encoding the immunomodulatory protein is inserted into the vector, are well known. Expression control sequences include promoters, activators, enhancers, operators, ribosomal binding sites, start signals, stop signals, cap signals, polyadenylation signals, and other signals involved with the control of transcription or translation. The promoter can be operably linked to the portion of the nucleic acid sequence encoding the immunomodulatory protein.

**[0549]** Transcriptional regulatory sequences include a promoter region sufficient to direct the initiation of RNA synthesis. Suitable eukaryotic promoters include the promoter of the mouse metallothionein I gene (Hamer et al, J. Molec. Appl Genet. 1:273 (1982)), the TK promoter of Herpes virus (McKnight, Cell 31:355 (1982)), the SV40 early promoter (Benoist et al, Nature 290:304 (1981)), the Rous sarcoma virus promoter (Gorman et al, Proc. Nat'l Acad. Sci. USA 79:6777 (1982)), the cytomegalovirus promoter (Foecking et al, Gene 45:101 (1980)), and the mouse mammary tumor virus promoter (see, generally, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in Protein Engineering: Principles and Practice, Cleland et al. (eds.), pages 163-181 (John Wiley & Sons, Inc. 1996)). One useful combination of a promoter and enhancer is provided by a myeloproliferative sarcoma virus promoter and a human cytomegalovirus enhancer.

**[0550]** Alternatively, a prokaryotic promoter, such as the bacteriophage T3 RNA polymerase promoter, can be used to control production of an immunomodulatory protein in mammalian cells if the prokaryotic promoter is regulated by a eukaryotic promoter (Zhou et al, Mol Cell. Biol. 10:4529 (1990), and Kaufman et al, Nucl. Acids Res. 19:4485 (1991)).

**[0551]** An expression vector can be introduced into host cells using a variety of standard techniques including calcium phosphate transfection, liposome-mediated transfection, microprojectile-mediated delivery, electroporation, and the like. The transfected cells can be selected and propagated to provide

recombinant host cells that comprise the expression vector stably integrated in the host cell genome. Techniques for introducing vectors into eukaryotic cells and techniques for selecting such stable transformants using a dominant selectable marker are described, for example, by Ausubel (1995) and by Murray (ed.), Gene Transfer and Expression Protocols (Humana Press 1991).

**[0552]** For example, one suitable selectable marker is a gene that provides resistance to the antibiotic neomycin. In this case, selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. 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. A suitable 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. Alternatively, markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

**[0553]** In some embodiments, polypeptides provided herein can also be made by synthetic methods. Solid phase synthesis is the preferred technique of making individual peptides since it is the most cost-effective method of making small peptides. For example, well known solid phase synthesis techniques include the use of protecting groups, linkers, and solid phase supports, as well as specific protection and deprotection reaction conditions, linker cleavage conditions, use of scavengers, and other aspects of solid phase peptide synthesis. Peptides can then be assembled into the polypeptides as provided herein.

## V. PHARMACEUTICAL COMPOSITIONS

**[0554]** Provided herein are compositions containing any of the provided immunomodulatory proteins described herein. The pharmaceutical composition can further comprise a pharmaceutically acceptable excipient. For example, the pharmaceutical composition can contain one or more excipients for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives.

**[0555]** In some embodiments, the pharmaceutical composition is a solid, such as a powder, capsule, or tablet. For example, the components of the pharmaceutical composition can be lyophilized. In some

embodiments, the solid pharmaceutical composition is reconstituted or dissolved in a liquid prior to administration.

**[0556]** In some embodiments, the pharmaceutical composition is a liquid, for example immunomodulatory proteins dissolved in an aqueous solution (such as physiological saline or Ringer's solution). In some embodiments, the pH of the pharmaceutical composition is between about 4.0 and about 8.5 (such as between about 4.0 and about 5.0, between about 4.5 and about 5.5, between about 5.0 and about 6.0, between about 5.5 and about 6.5, between about 6.0 and about 7.0, between about 6.5 and about 7.5, between about 7.0 and about 8.0, or between about 7.5 and about 8.5).

**[0557]** In some embodiments, the pharmaceutical composition comprises a pharmaceutically-acceptable excipient, for example a filler, binder, coating, preservative, lubricant, flavoring agent, sweetening agent, coloring agent, a solvent, a buffering agent, a chelating agent, or stabilizer. Examples of pharmaceutically-acceptable fillers include cellulose, dibasic calcium phosphate, calcium carbonate, microcrystalline cellulose, sucrose, lactose, glucose, mannitol, sorbitol, maltol, pregelatinized starch, corn starch, or potato starch. Examples of pharmaceutically-acceptable binders include polyvinylpyrrolidone, starch, lactose, xylitol, sorbitol, maltitol, gelatin, sucrose, polyethylene glycol, methyl cellulose, or cellulose. Examples of pharmaceutically-acceptable coatings include hydroxypropyl methylcellulose (HPMC), shellac, corn protein zein, or gelatin. Examples of pharmaceutically-acceptable disintegrants include polyvinylpyrrolidone, carboxymethyl cellulose, or sodium starch glycolate. Examples of pharmaceutically-acceptable lubricants include polyethylene glycol, magnesium stearate, or stearic acid. Examples of pharmaceutically-acceptable preservatives include methyl parabens, ethyl parabens, propyl paraben, benzoic acid, or sorbic acid. Examples of pharmaceutically-acceptable sweetening agents include sucrose, saccharine, aspartame, or sorbitol. Examples of pharmaceutically-acceptable buffering agents include carbonates, citrates, gluconates, acetates, phosphates, or tartrates.

**[0558]** In some embodiments, the pharmaceutical composition further comprises an agent for the controlled or sustained release of the product, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), beads, or liposomes.

**[0559]** In some embodiments, the pharmaceutical composition is sterile. Sterilization may be accomplished by filtration through sterile filtration membranes or radiation. Where the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

**[0560]** A pharmaceutically acceptable carrier may be a pharmaceutically acceptable material, composition, or vehicle. For example, the carrier may be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or some combination thereof. Each component of the carrier must be "pharmaceutically acceptable" in that it must be compatible with the other ingredients of the formulation. It also must be suitable for contact with any tissue, organ, or portion of the body that it may encounter, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

**[0561]** In some embodiments, the pharmaceutical composition is administered to a subject. Generally, dosages and routes of administration of the pharmaceutical composition are determined according to the size and condition of the subject, according to standard pharmaceutical practice. For example, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models such as mice, rats, rabbits, dogs, pigs, or monkeys. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. The exact dosage will be determined in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active compound or to maintain the desired effect. Factors that may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy.

**[0562]** Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation. The frequency of dosing will depend upon the pharmacokinetic parameters of the molecule in the formulation used. Typically, a composition is administered until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as multiple doses (at the same or different concentrations/dosages) over time, or as a continuous infusion. Further refinement of the appropriate dosage is routinely made. Appropriate dosages may be ascertained through use of appropriate dose-response data.

**[0563]** In some embodiments, the pharmaceutical composition is administered to a subject through any route, including orally, transdermally, by inhalation, intravenously, intra-arterially, intramuscularly, direct application to a wound site, application to a surgical site, intraperitoneally, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intrapleurally, intraventricularly, intra-articularly, intraocularly, or intraspinally.

**[0564]** A provided pharmaceutical formulation may, for example, be in a form suitable for intravenous infusion.

**[0565]** In some embodiments, the dosage of the pharmaceutical composition is a single dose or a repeated dose. In some embodiments, the doses are given to a subject once per day, twice per day, three times per day, or four or more times per day. In some embodiments, about 1 or more (such as about 2 or more, about 3 or more, about 4 or more, about 5 or more, about 6 or more, or about 7 or more) doses are given in a week. In some embodiments, multiple doses are given over the course of days, weeks, months, or years. In some embodiments, a course of treatment is about 1 or more doses (such as about 2 or more does, about 3 or more doses, about 4 or more doses, about 5 or more doses, about 7 or more doses, about 10 or more doses, about 15 or more doses, about 25 or more doses, about 40 or more doses, about 50 or more doses, or about 100 or more doses).

**[0566]** In some embodiments, an administered dose of the pharmaceutical composition is about 1 µg of protein per kg subject body mass or more (such as about 2 µg of protein per kg subject body mass or more, about 5 µg of protein per kg subject body mass or more, about 10 µg of protein per kg subject body mass or more, about 25 µg of protein per kg subject body mass or more, about 50 µg of protein per kg subject body mass or more, about 100 µg of protein per kg subject body mass or more, about 250 µg of protein per kg subject body mass or more, about 500 µg of protein per kg subject body mass or more, about 1 mg of protein per kg subject body mass or more, about 2 mg of protein per kg subject body mass or more, or about 5 mg of protein per kg subject body mass or more).

## VI. METHODS FOR ASSESSING ACTIVITY AND IMMUNE MODULATION OF IMMUNOMODULATORY PROTEINS

**[0567]** In some embodiments, the provided immunomodulatory proteins, such as BCMA fusion proteins or multi-domain immunomodulatory proteins provided herein, exhibit immunomodulatory activity. The provided immunodulatory proteins, such as BCMA fusion proteins or multi-domain immunomodulatory proteins, can modulate B cell activity, such as one or more of B cell proliferation, differentiation or survival. In some cases, the provided immunomodulatory proteins, such as multi-domain immunomodulatory proteins, may additionally modulate T cell activation or response. In some embodiments, T cell activation or response is reduced, decreased or attenuated.

**[0568]** The function of immunomodulatory proteins can be examined using a variety of approaches to assess the ability of the proteins to bind to cognate binding partners. For example, BCMA fusion proteins may be assessed for binding to APRIL or BAFF. In the case of multidomain immunomodulatory proteins herein the proteins may be assessed for binding to the cognate binding partner, such as a ligand of a T cell stimulatory receptor (e.g. CD80 or CD86) or directly to a T cell stimulatory receptor (e.g. CD28), and/or to a ligand of a B cell stimulatory receptor (e.g. APRIL or BAFF). A variety of assays are known for assessing binding affinity and/or determining whether a binding molecule (*e.g.*, immunomodulatory protein) specifically binds to a particular binding partner. It

is within the level of a skilled artisan to determine the binding affinity of a binding molecule, *e.g.*, immumodulaotry protein, for a binding partner, *e.g.*, APRIL or BAFF, such as by using any of a number of binding assays that are well known in the art. Various binding assays are known and include, but are not limited to, for example, ELISA $K_D$, KinExA, flow cytometry, and/or surface plasmon resonance devices), including those described herein. Such methods include, but are not limited to, methods involving BIAcore®, Octet®, or flow cytometry. For example, in some embodiments, a BIAcore® instrument can be used to determine the binding kinetics and constants of a complex between two proteins using surface plasmon resonance (SPR) analysis (*see*, *e.g.*, Scatchard *et al.*, *Ann. N.Y. Acad. Sci. 51*:660, 1949; Wilson, *Science 295*:2103, 2002; Wolff *et al.*, *Cancer Res. 53*:2560, 1993; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent). SPR measures changes in the concentration of molecules at a sensor surface as molecules bind to or dissociate from the surface. The change in the SPR signal is directly proportional to the change in mass concentration close to the surface, thereby allowing measurement of binding kinetics between two molecules. The dissociation constant for the complex can be determined by monitoring changes in the refractive index with respect to time as buffer is passed over the chip. Other suitable assays for measuring the binding of one protein to another include, for example, immunoassays such as enzyme linked immunosorbent assays (ELISA) and radioimmunoassays (RIA), or determination of binding by monitoring the change in the spectroscopic or optical properties of the proteins through fluorescence, UV absorption, circular dichroism, or nuclear magnetic resonance (NMR). Other exemplary assays include, but are not limited to, Western blot, ELISA, analytical ultracentrifugation, spectroscopy, flow cytometry, sequencing and other methods for detection of expressed polynucleotides or binding of proteins.

**[0569]** Provided immunomodulatory proteins also can be assessed in any of a variety of assess to assess modulation of T cell or B cell activity. One such assay is a cell proliferation assay. Cells are cultured in the presence or absence of a test compound (e.g. immunomodulatory protein), and cell proliferation is detected by, for example, measuring incorporation of tritiated thymidine or by colorimetric assay based on the metabolic breakdown of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Mosman, J. Immunol. Meth. 65: 55-63, 1983). An alternative assay format uses cells that are further engineered to express a reporter gene. The reporter gene is linked to a promoter element that is responsive to the receptor-linked pathway, and the assay detects activation of transcription of the reporter gene. Numerous reporter genes that are easily assayed for in cell extracts are known in the art, for example, the E. coli lacZ, chloroamphenicol acetyl transferase (CAT) and serum response element (SRE) (see, e.g., Shaw et al., Cell 56:563-72, 1989). An exemplary reporter gene is a luciferase gene (de Wet et al., Mol. Cell. Biol. 7:725, 1987). Expression of the luciferase gene is detected by luminescence using methods known in the art (e.g., Baumgartner et al., J. Biol. Chem. 269:29094-101,

1994; Schenborn and Goiffin, Promega Notes 41:11, 1993). Luciferase activity assay kits are commercially available from, for example, Promega Corp., Madison, Wis.

**[0570]** Provided immunomodulatory proteins can be characterized by the ability to inhibit the stimulation of human B cells by soluble APRIL or BAFF, as described by Gross et αl, international publication No. WO00/40716. Briefly, human B cells are isolated from peripheral blood mononuclear cells, such as using CD19 magnetic beads separation (e.g. Miltenyi Biotec Auburn, CA). The purified B cells can be incubated under conditions of stimulation, e.g. in the presence of soluble APRIL, and further in the presence of titrated concentration of immunomodulatory protein. The B cells can be labeled with a proliferation dye or can be labeled with 1 μCi $^{3}$H-thymidine to measure proliferation. The number of B cells can be determined over time.

**[0571]** Reporter cell lines that express a reporter gene under the operable control of a transcription factor, such as NF-κB, NFAT-1 and AP-1, can be made that express TACI or BCMA. For example, the reporter cell can include Jurkat and other B Lymphoma cell lines. Incubation of these cells with soluble BAFF or APRIL ligands signal through the reporter genes in these constructs. The effect of provided immunomodulatory proteins to modulate this signaling can be assessed.

**[0572]** Well established animal models are available to test in vivo efficacy of provided immunomodulatory proteins in certain disease states, including those involving autoimmune or inflammatory conditions. For example, animal models of autoimmune disease include, for example, MRL-lpr/lpr or NZB×NZW F1 congenic mouse strains which serve as a model of SLE (systemic lupus erythematosus). Such animal models are known in the art, see for example *Autoimmune Disease Models* A Guidebook, Cohen and Miller eds. Academic Press. Offspring of a cross between New Zealand Black (NZB) and New Zealand White (NZW) mice develop a spontaneous form of SLE that closely resembles SLE in humans. The offspring mice, known as NZBW begin to develop IgM autoantibodies against T-cells at 1 month of age, and by 5-7 months of age, Ig anti-DNA autoantibodies are the dominant immunoglobulin. Polyclonal B-cell hyperactivity leads to overproduction of autoantibodies. The deposition of these autoantibodies, particularly ones directed against single stranded DNA is associated with the development of glomerulonephritis, which manifests clinically as proteinuria, azotemia, and death from renal failure. Kidney failure is the leading cause of death in mice affected with spontaneous SLE, and in the NZBW strain, this process is chronic and obliterative. The disease is more rapid and severe in females than males, with mean survival of only 245 days as compared to 406 days for the males. While many of the female mice will be symptomatic (proteinuria) by 7-9 months of age, some can be much younger or older when they develop symptoms. The fatal immune nephritis seen in the NZBW mice is very similar to the glomerulonephritis seen in human SLE, making this spontaneous murine model very attractive for testing of potential SLE therapeutics (Putterman and Naparstek, *Murine Models of Spontaneous Systemic Lupus Erythematosus*, Autoimmune Disease Models: A Guidebook,

chapter 14, pp. 217-34, 1994; Mohan et al., *J. Immunol.* 154:1470-80, 1995; and Daikh et al., *J. Immunol.* 159:3104-08, 1997). Administration of provided immunomodulatory proteins to these mice to evaluate the efficacy to ameliorate symptoms and alterations to the course of disease can be assessed.

**[0573]** Another mouse model of inflammation and lupus-like disease is the bm12 inducible mouse model of SLE (Klarquist and Janssen, 2015. J. Vis. Exp. (105), e53319). Splenocyte suspensions from female I-A$^{bm12}$B6(C)-*H2*-Ab1$^{bm12}$/KhEgJ ('bm12') mice are adoptively transferred into female C57BL/6NJ recipient mice. H2-Ab1$^{bm12}$ differs from H2-Ab1$^{b}$ by *3* nucleotides, resulting in alteration of 3 amino acids in the β-chain of the MHC class II I-A molecule. Alloactivation of donor bm12 CD4+ T cells by recipient antigen presenting cells leads to chronic GVHD with symptoms closely resembling SLE, including autoantibody production, changes in immune cell subsets, and mild kidney disease. Glomerulonephritis with immune complex deposition develops late in the model, largely comprised of autoantigens bound to IgG1, IgG2b, IgG2c, and IgG3 antibodies. Endpoints of this model may include concentrations of anti-dsDNA antibodies, select IgG isotypes, blood urea nitrogen (BUN), and creatinine in serum, immune cell subset composition in the spleen and cervical LN, and kidney histology.

**[0574]** In some embodiments, mouse models for Sjögren's syndrome (SjS) can be used. The SjS disease, as well as an accelerated onset of diabetes, can be induced in female diabetes-prone non-obese diabetic (NOD) mice using repeat dosing with anti-mouse (m) PD-L1 antibody, based on a modified version of a protocol published by Zhou et al., 2016 Sci. Rep. 6, 39105. Starting at 6 weeks of age, mice are injected intraperitoneally (IP) on Study Days 0, 2, 4, and 6 with 100 µg of anti-PD-L1 antibody and are treated on various days with provided immunomodulatory proteins. Naïve mice are included as controls for the endpoint analyses. All mice are typically terminated on Study Day 10 and submandibular glands (SMG) and the pancreas from each mouse are collected for histopathology evaluation to assess for signs and severity of sialadenitis and insulitis. Blood glucose levels can be measured on various days.

**[0575]** In some embodiments, mouse models for experimental allergic encephalomyelitis (EAE) can be used. The models resemble human multiple sclerosis, and produces demyelination as a result of T-cell activation to neuroproteins such as myelin basic protein (MBP), or proteolipid protein (PLP). Inoculation with antigen leads to induction of CD4+, class II MHC-restricted T-cells (Th1). Changes in the protocol for EAE can produce acute, chronic-relapsing, or passive-transfer variants of the model (Weinberg et al., *J. Immunol.* 162:1818-26, 1999; Mijaba et al., *Cell. Immunol.* 186:94-102, 1999; and Glabinski, *Meth. Enzym.* 288:182-90, 1997). Administration of provided immunomodulatory proteins to ameliorate symptoms and alterations to the course of disease can be assessed.

**[0576]** In some embodiments, a collagen-induced arthritis (CIA) model can be used in which mice develop chronic inflammatory arthritis which closely resembles human rheumatoid arthritis (RA). Since CIA shares similar immunological and pathological features with RA, this makes it an ideal model for screening potential human anti-inflammatory compounds. Another advantage in using the CIA model is

that the mechanisms of pathogenesis are known. The T and B cell epitopes on type II collagen have been identified, and various immunological (delayed-type hypersensitivity and anti-collagen antibody) and inflammatory (cytokines, chemokines, and matrix-degrading enzymes) parameters relating to immune-mediating arthritis have been determined, and can be used to assess test compound efficacy in the models (Wooley, *Curr. Opin. Rheum.* 3:407-20, 1999; Williams et al., *Immunol.* 89:9784-788, 1992; Myers et al., *Life Sci.* 61:1861-78, 1997; and Wang et al., *Immunol.* 92:8955-959, 1995). Administration of provided immunomodulatory proteins to ameliorate symptoms and alterations to the course of disease can be assessed.

**[0577]** In some embodiments, models for bronchial infection, such as asthma, can be created when mice are injected with ovalbumin and restimulated nasally with antigen which produces an asthmatic response in the bronchi similar to asthma. Administration of provided immunomodulatory proteins to ameliorate symptoms and alterations to the course of disease can be assessed..

**[0578]** In some embodiments, myasthenia gravis (MG) is another autoimmune disease for which murine models are available. MG is a disorder of neuromuscular transmission involving the production of autoantibodies directed against the nicotinic acetylcholine receptor (AChR). MG is acquired or inherited with clinical features including abnormal weakness and fatigue on exertion. A mouse model of MG has been established. (Christadoss et al., *Establishment of a Mouse Model of Myasthenia Gravis Which Mimics Human Myasthenia Gravis Pathogenesis for Immune Intervention*, in Immunobiology of Proteins and Peptides VIII, Atassi and Bixler, eds., 1995, pp. 195-99.) Experimental autoimmune myasthenia gravis (EAMG) is an antibody mediated disease characterized by the presence of antibodies to AChR. These antibodies destroy the receptor leading to defective neuromuscular electrical impulses, resulting in muscle weakness. In the EAMG model, mice are immunized with the nicotinic acetylcholine receptor. Clinical signs of MG become evident weeks after the second immunization. EAMG is evaluated by several methods including measuring serum levels of AChR antibodies by radioimmunoassay (Christadoss and Dauphinee, *J. Immunol.* 136:2437-40, 1986; and Lindstrom et al., *Methods Enzymol.* 74:432-60, 1981), measuring muscle AChR, or electromyography (Wu et al. *Protocols in Immunology.* Vol. 3, Eds. Coligen, Kruisbeak, Margulies, Shevach, and Strober. John Wiley and Sons, New York, p. 15.8.1, 1997).

**[0579]** Another use for in vivo models includes delivery of an antigen challenge to the animal followed by administration of immunomodulatory proteins and measuring the T and B cell response. T cell dependent and T cell independent immune response can be measured as described in Perez-Melgosa et al., *J. Immunol.* 163:1123-7, 1999. Immune response in animals subjected to a regular antigen challenge (for example, keyhole limpet hemacyanin (KLH), sheep red blood cells (SRBC), ovalbumin or collagen) followed by administration of provided immunomodulatory proteins can be done to measure effect on B cell response.

**[0580]** Pharmacokinetic studies can be used in association with radiolabeled immunomodulatory proteins to determine the distribution and half life of such polypeptides in vivo.

**[0581]** Assays to assess activity on T cell responses also can be assessed. T cell activation assays can be employed in which IFN-gamma or other effector cytokine is measured. The immunomodulatory proteins can be assessed for their ability to suppress or decrease effector cytokine secretion following T cell activation. Assays for determining enhancement or suppression of immunological activity include MLR (mixed lymphocyte reaction) assays measuring interferon-gamma cytokine levels in culture supernatants (Wang et al., Cancer Immunol Res. 2014 Sep: 2(9):846-56), SEB (staphylococcal enterotoxin B), T cell stimulation assays (Wang et al., Cancer Immunol Res. 2014 Sep: 2(9):846-56), and anti-CD3 T cell stimulation assays (Li and Kurlander, J Transl Med. 2010: 8: 104). Since T cell activation is associated with secretion of IFN-gamma cytokine, detecting IFN-gamma levels in culture supernatants from these in vitro human T cell assays can be assayed using commercial ELISA kits (Wu et al, Immunol Lett 2008 Apr 15; 117(1): 57-62). Assays also include assays to assess cytotoxicity, including a standard 51Cr-release assay (see e.g. Milone et al., (2009) Molecular Therapy 17: 1453-1464) or flow based cytotoxicity assays, or an impedance based cytotoxicity assay (Peper et al. (2014) Journal of Immunological Methods, 405:192-198). In some embodiments, the assay used is anti-CD3 coimmobilization assay. In this assay, primary T cells are stimulated by anti-CD3 immobilized with or without additional recombinant proteins. Culture supernatants are harvested at timepoints, usually 24-72 hours. In another embodiment, the assay used is a mixed lymphocyte reaction (MLR). In this assay, primary T cells are simulated with allogenic APC. Culture supernatants are harvested at timepoints, usually 24-72 hours. Human IFN-gamma levels are measured in culture supernatants by standard ELISA techniques. In some cases, commercial kits are available from vendors and the assay can be performed according to manufacturer's recommendation.

**[0582]** In some embodiments, in assaying for the ability of an immunomodulatory protein to modulate, e.g. increase or decrease IFN-gamma expression, a T cell reporter assay can be used. In some embodiments, the T cell is a Jurkat T cell line or is derived from Jurkat T cell lines. In reporter assays, the reporter cell line (e.g. Jurkat reporter cell) also is generated to overexpress a receptor that is the cognate binding partner of the immunomodulatory protein. In some embodiments, the reporter T cells also contain a reporter construct containing an inducible promoter responsive to T cell activation operably linked to a reporter. In some embodiments, the reporter is a fluorescent or luminescent reporter. In some embodiments, the reporter is luciferase. In some embodiments, the promoter is responsive to CD3 signaling. In some embodiments, the promoter is an NFAT promoter. In some embodiments, the promoter is responsive to costimulatory signaling, e.g. CD28 costimulatory signaling. In some embodiments, the promoter is an IL-2 promoter. In aspects of a reporter assay, a reporter cell line is stimulated, such as by co-incubation with antigen presenting cells (APCs) expressing the wild-type

ligand of a T cell costimulatory receptor. In some embodiments, the APCs are artificial APCs. Artificial APCs are well known to a skilled artisan. In some embodiments, artificial APCs are derived from one or more mammalian cell line, such as K562, CHO or 293 cells.

## VII. THERAPEUTIC APPLICATIONS

**[0583]** The pharmaceutical compositions described herein (including pharmaceutical composition comprising the immunomodulatory protein described herein) can be used in a variety of therapeutic applications, such as the treatment of a disease. For example, in some embodiments the pharmaceutical composition is used to treat inflammatory or autoimmune disorders, cancer, organ transplantation, viral infections, and/or bacterial infections in a mammal. The pharmaceutical composition can modulate (e.g. decrease) an immune response to treat the disease.

**[0584]** Such methods and uses include therapeutic methods and uses, for example, involving administration of the molecules or compositions containing the same, to a subject having a disease, condition, or disorder. In some cases, such as described, the disease or disorder is an autoimmune or inflammatory disease, condition or disorder. In some embodiments, the molecule or engineered cell is administered in an effective amount to effect treatment of the disease, condition or disorder. Uses include uses of molecules containing an immunomodulatory protein, and in the preparation of a medicament in order to carry out such therapeutic methods. In some embodiments, the methods are carried out by administering a provided immunomodulatory protein, or compositions comprising the same, to the subject having or suspected of having the disease or condition. In some embodiments, the methods thereby treat the disease or condition or disorder in the subject.

**[0585]** Illustrative subjects include mammalian subjects, such as farm animals, domestic animals, and human patients. In particular embodiments, the subject is a human subject.

**[0586]** The pharmaceutical compositions described herein can be used in a variety of therapeutic applications, such as the treatment of a disease. For example, in some embodiments the pharmaceutical composition is used to treat inflammatory or autoimmune disorders, organ transplantation, viral infections, and/or bacterial infections in a mammal. The pharmaceutical composition can modulate an immune response to treat the disease. In some embodiments, the pharmaceutical composition suppresses an immune response, which can be useful in the treatment of inflammatory or autoimmune disorders, or organ transplantation.

**[0587]** The provided methods are believed to have utility in a variety of applications, including, but not limited to, e.g., in prophylactic or therapeutic methods for treating a variety of immune system diseases or conditions in a mammal in which modulation or regulation of the immune system and immune system responses is beneficial. For example, suppressing an immune response can be beneficial in prophylactic and/or therapeutic methods for inhibiting rejection of a tissue, cell, or organ transplant

from a donor by a recipient. In a therapeutic context, the mammalian subject is typically one with an immune system disease or condition, and administration is conducted to prevent further progression of the disease or condition.

**[0588]** The provided immunomodulatory proteins, including BCMA fusion proteins and multi-domain immunomodulatory proteins, can be used for the treatment of autoimmune diseases, B cell cancers, immunomodulation, EBD and any antibody- mediated pathologies (e.g., ITCP, myasthenia gravis and the like), renal diseases, indirect T cell immune response, graft rejection, and graft versus host disease. Administration of the immunomodulatory proteins can specifically regulate B cell responses during the immune response. Additionally, administration of provided immunomodulatory proteins can be used to modulate B cell development, development of other cells, antibody production, and cytokine production. Administration or use of provided immunomodulatory proteins can also modulate T and B cell communication, such as by neutralizing the proliferative effects of BAFF or APRIL alone or, in the case of provided multi-domain immunomodulatory proteins also by neutralizing proliferative effects mediated by T cell stimulatory molecules such as by neutralizing the proliferative effects of CD80/CD86 on CD28.

**[0589]** In some embodiments, the pharmaceutical composition suppresses an immune response, which can be useful in the treatment of inflammatory or autoimmune disorders, or organ transplantation. In some embodiments, the pharmaceutical composition contains an immunomodulatory proteins that exhibits antagonist activity of a B cell stimulatory receptor and/or T cell stimulatory receptor, thereby decreasing or reducing an immune response.

**[0590]** In some embodiments, the compositions can be used to treat an autoimmune disease. In some embodiments, the administration of a therapeutic composition containing an immunomodulatory protein provided herein to a subject suffering from an immune system disease (e.g., autoimmune disease) can result in suppression or inhibition of such immune system attack or biological responses associated therewith. By suppressing this immune system attack on healthy body tissues, the resulting physical symptoms (e.g., pain, joint inflammation, joint swelling or tenderness) resulting from or associated with such attack on healthy tissues can be decreased or alleviated, and the biological and physical damage resulting from or associated with the immune system attack can be decreased, retarded, or stopped. In a prophylactic context, the subject may be one with, susceptible to, or believed to present an immune system disease, disorder or condition, and administration is typically conducted to prevent progression of the disease, disorder or condition, inhibit or alleviate symptoms, signs, or biological responses associated therewith, prevent bodily damage potentially resulting therefrom, and/or maintain or improve the subject's physical functioning.

**[0591]** In some embodiments, the disease or conditions that can be treated by the pharmaceutical composition described herein is any disease mediated by immune complex deposition (e.g. lupus

nephritis, vasculitis); direct interference with a pathway (e.g. catastrophic antiphospholipid antibody syndrome, myasthenia gravis crisis; anti-Jo-1 disease); opsonization or direct damage to cells (e.g. Idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia); antibody-mediated rejection of an allograft (e.g. highly-sensitized renal transplant patients); or anti-drug antibodies to biologic replacement factors, vectors (e.g. anti-Factor 8).

**[0592]** In some embodiments, the inflammatory and autoimmune disorders that can be treated by the pharmaceutical composition described herein is Systemic lupus erythematosus (SLE), including flare prevention without glucocorticoids; Sjögren's syndrome; Primary biliary cirrhosis (PBC); Systemic scleroderma; Polymyositis; Diabetes prevention; IgA nephropathy; IgA vasculitis; B cell cancers, for example myeloma; Multiple sclerosis or Optic neuritis.

**[0593]** In some embodiments, the provided immunomodulatory proteins can be used to treat pre-B or B-cell leukemias, such as plasma cell leukemia, chronic or acute lymphocytic leukemia, myelomas such as multiple myeloma, plasma cell myeloma, endothelial myeloma and giant cell myeloma, and lymphomas such as non-Hodgkins lymphoma.

**[0594]** In some embodiments, the provided immunomodulatory proteins can be used as immunosuppressants to selectively block the action of B-lymphocytes for use in treating disease. For example, certain autoimmune diseases are characterized by production of autoantibodies, which contribute to tissue destruction and exacerbation of disease. Autoantibodies can also lead to the occurrence of immune complex deposition complications and lead to many symptoms of systemic lupus erythematosus, including kidney failure, neuralgic symptoms and death. Modulating antibody production independent of cellular response would also be beneficial in many disease states. B cells have also been shown to play a role in the secretion of arthritogenic immunoglobulins in rheumatoid arthritis. Methods and uses of the provided immunomodulatory proteins to inhibit, block or neutralize action of B cell cells to thereby suppress antibody production would be beneficial in treatment of autoimmune diseases such as myasthenia gravis, rheumatoid arthritis, polyarticular-course juvenile rheumatoid arthritis, and psoriatic arthritis.

**[0595]** In some embodiments, the provided immunomodulatory proteins can be used to block or neutralize the actions of B-cells in association with end stage renal diseases, which may or may not be associated with autoimmune diseases. Such methods would also be useful for treating immunologic renal diseases. Such methods would be useful for treating glomerulonephritis associated with diseases such as membranous nephropathy, IgA nephropathy or Berger's Disease, IgM nephropathy, IgA Vasculitis, Goodpasture's Disease, post-infectious glomerulonephritis, mesangioproliferative disease, chronic lymphoid leukemia, minimal-change nephrotic syndrome. Such methods would also serve as therapeutic applications for treating secondary glomerulonephritis or vasculitis associated with such diseases as lupus, polyarteritis, Henoch-Schonlein, Scleroderma, HTV-related diseases, amyloidosis or hemolytic

uremic syndrome. The provided methods would also be useful as part of a therapeutic application for treating interstitial nephritis or pyelonephritis associated with chronic pyelonephritis, analgesic abuse, nephrocalcinosis, nephropathy caused by other agents, nephrolithiasis, or chronic or acute interstitial nephritis. The methods provided herein also include use of the provided immunomodulatory proteins in the treatment of hypertensive or large vessel diseases, including renal artery stenosis or occlusion and cholesterol emboli or renal emboli. The provided methods and uses also can be used for treatment of renal or urological neoplasms, multiple myelomas, lymphomas, light chain neuropathy or amyloidosis.

**[0596]** In some embodiments, the provided immunomodulatory proteins also can be used for the treatment of asthma and other chronic airway diseases such as bronchitis and emphysema. The provided immunomodulatory proteins can also be used to treat Sjogren's Syndrome.

**[0597]** In some embodiments, methods and uses of the provided immunomodulatory proteins include immunosuppression, in particular for such therapeutic use as for graft-versus-host disease and graft rejection. In some embodiments, methods and uses of the provided immunomodulatory proteins include treatment of such autoimmune diseases as insulin dependent diabetes mellitus (IDDM) and Crohn's Disease. Methods provided herein would have additional therapeutic value for treating chronic inflammatory diseases, in particular to lessen joint pain, swelling, anemia and other associated symptoms as well as treating septic shock.

**[0598]** In some embodiments, the inflammatory and autoimmune disorders that can be treated by a pharmaceutical composition containing an immunomodulatory protein described herein include, but are not limited to, Achalasia; Addison’s disease; Adult Still's disease; Agammaglobulinemia; Alopecia areata; Amyloidosis; Ankylosing spondylitis; Anti-GBM/Anti-TBM nephritis; Antiphospholipid syndrome; Autoimmune adrenalitis (Addison's disease); Autoimmune angioedema; Autoimmune dysautonomia; Autoimmune encephalomyelitis; Autoimmune hepatitis; Autoimmune inner ear disease (AIED); Autoimmune myocarditis; Autoimmune oophoritis; Autoimmune orchitis; Autoimmune pancreatitis; Autoimmune polyglandular syndrome type II (APS II); Autoimmune retinopathy; Autoimmune thyroid disease (AITD), i.e. Hashimoto’s disease; Autoimmune urticarial; Axonal & neuronal neuropathy (AMAN); Baló disease; Behcet’s disease; Benign mucosal pemphigoid; Bullous pemphigoid; Castleman disease (CD); Celiac disease; Chagas disease; Chronic inflammatory demyelinating polyneuropathy (CIDP); Chronic recurrent multifocal osteomyelitis (CRMO); Churg-Strauss Syndrome (CSS) or Eosinophilic Granulomatosis (EGPA); Cicatricial pemphigoid; Cogan’s syndrome; Cold agglutinin disease; Congenital heart block; Coxsackie myocarditis; CREST syndrome; Crohn’s disease; Dermatitis herpetiformis; Dermatomyositis; Devic’s disease (neuromyelitis optica); Discoid lupus; Dressler’s syndrome; Endometriosis; Eosinophilic esophagitis (EoE); Eosinophilic fasciitis; Erythema nodosum; Essential mixed cryoglobulinemia; Evans syndrome; Fibromyalgia; Fibrosing alveolitis; Giant cell arteritis (temporal arteritis); Giant cell myocarditis; Glomerulonephritis;

Goodpasture's syndrome; Granulomatosis with Polyangiitis; Graves' disease; Guillain-Barre syndrome; Hashimoto's thyroiditis; Hemolytic anemia; Henoch-Schonlein purpura (HSP); Herpes gestationis or pemphigoid gestationis (PG); Hidradenitis Suppurativa (HS) (Acne Inversa); Hypogammalglobulinemia; IgA Nephropathy; IgA Vasculitis; IgG4-related sclerosing disease; Immune thrombocytopenic purpura (ITP); Inclusion body myositis (IBM); Interstitial cystitis (IC); Juvenile arthritis; Juvenile diabetes (Type 1 diabetes); Juvenile myositis (JM); Kawasaki disease; Lambert-Eaton syndrome; Leukocytoclastic vasculitis; Lichen planus; Lichen sclerosus; Ligneous conjunctivitis; Linear IgA disease (LAD); Lupus; Lyme disease chronic; Meniere's disease; Microscopic polyangiitis (MPA); Mixed connective tissue disease (MCTD); Mooren's ulcer; Mucha-Habermann disease; Multifocal Motor Neuropathy (MMN) or MMNCB; Multiple sclerosis; Myasthenia gravis; Myositis; Narcolepsy; Neonatal Lupus; Neuromyelitis optica; Neutropenia; Ocular cicatricial pemphigoid; Optic neuritis; Palindromic rheumatism (PR); PANDAS; Paraneoplastic cerebellar degeneration (PCD); Paroxysmal nocturnal hemoglobinuria (PNH); Parry Romberg syndrome; Pars planitis (peripheral uveitis); Parsonage-Turner syndrome; Pemphigus, Pemphigus vulgaris; Peripheral neuropathy; Perivenous encephalomyelitis; Pernicious anemia (PA); POEMS syndrome; Polyarteritis nodosa; Polyglandular syndromes type I, II, III; Polymyalgia rheumatic; Polymyositis; Postmyocardial infarction syndrome; Postpericardiotomy syndrome; Primary biliary cirrhosis; Primary sclerosing cholangitis; Progesterone dermatitis; Psoriasis; Psoriatic arthritis; Pure red cell aplasia (PRCA); Pyoderma gangrenosum; Raynaud's phenomenon; Reactive Arthritis; Reflex sympathetic dystrophy; Relapsing polychondritis; Restless legs syndrome (RLS); Retroperitoneal fibrosis; Rheumatic fever; Rheumatoid arthritis; Sarcoidosis; Schmidt syndrome; Scleritis; Scleroderma; Sjögren's syndrome; Sperm & testicular autoimmunity; Stiff person syndrome (SPS); Subacute bacterial endocarditis (SBE); Susac's syndrome; Sympathetic ophthalmia (SO); Takayasu's arteritis; Temporal arteritis/Giant cell arteritis; Thrombocytopenic purpura (TTP); Tolosa-Hunt syndrome (THS); Transverse myelitis; Type 1 diabetes; Ulcerative colitis (UC); Undifferentiated connective tissue disease (UCTD); Uveitis; Vasculitis; Vitiligo or Vogt-Koyanagi-Harada Disease.

**[0599]** In some embodiments, the provided immunomodulatory proteins, including BCMA-Fc fusion proteins and provided multi-domain immunomodulatory (e.g. TIM-BIM) fusion proteins, can be used to treat Scleroderma, Myasthenia gravis, GVHD (including acute GVHD or chronic GVHD), an immune response in connection with transplantation; Antiphospholipid Ab syndrome; Multiple sclerosis; Sjogren's syndrome; IgG4-related disease; Type I diabetes; Rheumatoid arthritis including glucocorticoid therapy (GC) RA or Acute lupus nephritis.

**[0600]** In some embodiments, the provided immunomodulatory proteins, including BCMA-Fc fusion proteins and provided multi-domain immunomodulatory (e.g. TIM-BIM) fusion proteins, can be used to treat Amyotrophic lateral sclerosis, Neuromyelitis optica, Transverse myelitis, CNS autoimmunity, Guillain-barre syndrome, Neurocystercercosis, Sarcoidosis (T/seroneg), Churg-Strauss

Syndrome, Hashimoto's thyroiditis, Grave's disease, immune thrombocytopenia (ITP), Addison's Disease, Polymyositis, or Dermatomyositis.

**[0601]** In some embodiments, the provided immunomodulatory proteins, including BCMA-Fc fusion proteins and provided multi-domain immunomodulatory (e.g. TIM-BIM) fusion proteins, can be used to treat IgA nephropathy, chronic inflammatory demyelinating polyneuropathy (CIDP), antisynthetase disease such as Jo-1 syndrome, or ANCA vasculitis.

**[0602]** In some embodiments, the provided immunomodulatory proteins, including BCMA-Fc fusion proteins and provided multi-domain immunomodulatory (e.g. TIM-BIM) fusion proteins, can be used to treat a B cell cancer. In some embodiments, the B cell cancer is a cancer in which BAFF and APRIL are involved or implicated in providing an autocrine survival loop to the B cells. In some embodiments, the cancer is B cell chronic lymphocytic leukemia, non-Hodgkins' lymphoma or myeloma. In some embodiments, the cancer is myeloma.

**[0603]** In some embodiments, a therapeutic amount of the pharmaceutical composition is administered. Typically, precise amount of the compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, extent of infection, and condition of the patient (subject). The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

**[0604]** The administration of the subject compositions may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In one embodiment, the therapeutic composition is administered to a patient by intradermal or subcutaneous injection. In another embodiment, the therapeutic composition is administered by i.v. injection.

**[0605]** In some embodiments, the pharmaceutical composition is administered as a monotherapy (i.e., as a single agent) or as a combination therapy (i.e., in combination with one or more additional immunosuppressant agents). In some embodiments, the additional agent is a glucocorticoid (e.g., prednisone, dexamethasone, and hydrocortisone), cytostatic agent, such as a cytostatic agent that affect proliferation of T cells and/or B cells (e.g., purine analogs, alkylating agents, or antimetabolites), an antibody (e.g., anti-CD20, anti-CD25 or anti-CD3 monoclonal antibodies), cyclosporine, tacrolimus, sirolimus, everolimus, an interferon, an opiod, a TNF binding protein, mycophenolate, small biological agent, such as fingolimod or myriocin, cytokine, such as interferon beta-1a, an integrin agonist, or an integrin antagonist.

## VIII. ARTICLES OF MANUFACTURE AND KITS

**[0606]** Also provided herein are articles of manufacture that comprise the pharmaceutical compositions described herein in suitable packaging. Suitable packaging for compositions (such as ophthalmic compositions) described herein are known in the art, and include, for example, vials (such as sealed vials), vessels, ampules, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

**[0607]** Further provided are kits comprising the pharmaceutical compositions (or articles of manufacture) described herein, which may further comprise instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may also include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein.

## IX. EXEMPLARY EMBODIMENTS

**[0608]** Among the provided embodiments are:

1. An immunomodulatory protein comprising:

(1) at least one T cell inhibitory molecule (TIM) that binds to (i) a T cell stimulatory receptor, or (ii) a ligand of a T cell stimulatory receptor; and/or that antagonizes activity of a T cell stimulatory receptor; and

(2) at least one B cell inhibitory molecule (BIM) that binds to a ligand of a B cell stimulatory receptor and/or antagonizes activity of a B cell stimulatory receptor.

2. The immunomodulatory protein of embodiment 1, wherein the TIM binds to a ligand of a T cell stimulatory receptor.

3. The immunomodulatory protein of embodiment 2, wherein:

the T cell stimulatory receptor is CD28; and/or

the ligand of the T cell stimulatory receptor is CD80 or CD86.

4. The immunomodulatory protein of any of embodiments 1-3, wherein the TIM is a CTLA-4 extracellular domain or a binding portion thereof that binds to CD80 or CD86.

5. The immunomodulatory protein of embodiment 4, wherein the CTLA-4 extracellular domain or binding portion thereof consists of (i) the sequence of amino acids set forth in SEQ ID NO:1 or SEQ ID NO:2, (ii) a variant CTLA-4 sequence of amino acids that has at least 85% sequence identity to SEQ ID NO:1 or SEQ ID NO:2; or (iii) a portion thereof comprising an IgV domain.

6. The immunomodulatory protein of embodiment 4 or embodiment 5, wherein the CTLA-4 extracellular domain consists of the sequence of amino acids set forth in SEQ ID NO:1.

7. The immunomodulatory protein of embodiment 4 or embodiment 5, wherein the CTLA-4 extracellular domain consists of a variant CTLA-4 sequence of amino acids that has at least 85%

sequence identity to SEQ ID NO:1 or a portion thereof comprising an IgV domain, wherein the variant sequence comprises one or more amino acid substitutions in SEQ ID NO:1 or the portion thereof comprising the IgV domain.

8. The immunomodulatory protein of embodiment 7, wherein the variant CTLA-4 binds to the ectodomain of CD80 and CD86, optionally wherein binding affinity to one or both of CD80 and CD86 is increased compared to the sequence set forth in SEQ ID NO:1 or the portion thereof comprising the IgV domain.

9. The immunomodulatory protein of embodiment 8, wherein the variant CTLA-4 consists of the sequence set forth in SEQ ID NO:92 or a portion thereof comprising the IgV domain.

10. The immunomodulatory protein of embodiment 8, wherein the variant CTLA-4 consists of the sequence set forth in SEQ ID NO:113 or a portion thereof comprising the IgV domain.

11. The immunomodulatory protein of embodiment 8, wherein the variant CTLA-4 consists of the sequence set forth in SEQ ID NO:165 or a portion thereof comprising the IgV domain.

12. The immunomodulatory protein of embodiment 8, wherein the variant CTLA-4 consists of the sequence set forth in SEQ ID NO:186 or a portion thereof comprising the IgV domain.

13. The immunomodulatory protein of any of embodiments 1-12, wherein:
the ligand of a B cell stimulatory receptor is APRIL or BAFF; and/or
the B cell stimulatory receptor is TACI, BCMA, or BAFF-receptor.

14. The immunomodulatory protein of any of embodiments 1-13, wherein the BIM is a TACI polypeptide that consists of the TACI extracellular domain or a binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

15. The immunomodulatory protein of embodiment 14, wherein the TACI polypeptide is a truncated wild-type TACI extracellular domain or is a variant thereof, wherein the truncated wild-type TACI extracellular domain contains the cysteine rich domain 2 (CRD2) but lacks the entirety of the cysteine rich domain 1 (CRD1), wherein the variant TACI polypeptide comprises one or more amino acid substitutions in the truncated wild-type TACI extracellular domain.

16. The immunomodulatory protein of embodiment 14 or embodiment 15, wherein the TACI polypeptide is a truncated wild-type TACI extracellular domain or is a variant thereof, wherein the truncated wild-type TACI extracellular domain consists of a contiguous sequence contained within amino acid residues 67-118 that consists of amino acid residues 71-104, with reference to positions set forth in SEQ ID NO:709, wherein the variant TACI polypeptide comprises one or more amino acid substitutions in the truncated wild-type TACI extracellular domain.

17. The immunomodulatory protein of any of embodiments 1-13, wherein the BIM is a BCMA polypeptide that consists of the BCMA extracellular domain or a binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

18. The immunomodulatory protein of any of embodiments 14-16, , wherein the truncated wild-type TACI extracellular domain is 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 59, 50 or 51 amino acids in length.

19. The immunomodulatory protein of any of embodiments 14-16 and 17, wherein the truncated wild-type TACI extracellular domain consists of amino acid residues 68-110 set forth in SEQ ID NO: 709.

20. The immunomodulatory protein of any of embodiments 14-16 and 17-19, wherein the TACI polypeptide consists of the sequence of amino acid set forth in SEQ ID NO:528 or is a variant thereof containing one or more amino acid substitutions in the sequence set forth in SEQ ID NO:528.

21. The immunomodulatory protein of any of embodiments 14-16 and 17-20, wherein the truncated TACI polypeptide or the variant thereof binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

22. The immunomodulatory protein of any of embodiments 14-16, wherein the TACI polypeptide is a truncated wild-type TACI extracellular domain that consists of the sequence set forth in SEQ ID NO: 516.

23. The immunomodulatory protein of any of embodiments 14-16 and 17-21, wherein the TACI polypeptide is a truncated wild-type TACI extracellular domain that consists of the sequence set forth in SEQ ID NO:528.

24. The immunomodulatory protein of any of embodiments 15-16 and 17-21, wherein the TACI polypeptide is the variant TACI polypeptide, wherein the variant TACI polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the truncated TACI polypeptide.

25. The immunomodulatory protein of any of embodiments 15-16, 17-21 and 24, wherein the variant TACI polypeptide comprises one or more amino acid substitutions at positions selected from among 74, 75, 76, 77, 78, 79, 82, 83, 84, 85, 86, 87, 88, 92, 95, 97, 98, 99, 101, 102 and 103, corresponding to numbering set forth in SEQ ID NO:709.

26. The immunomodulatory protein of embodiment 25, wherein the one or more amino acid substitutions are selected from E74V, Q75E, Q75R, G76S, K77E, F78Y, Y79F, L82H, L82P, L83S, R84G, R84L, R84Q, D85E, D85V, C86Y, I87L, I87M, S88N, I92V, Q95R, P97S, K98T, Q99E, A101D, Y102D, F103S, F103V, F103Y, or a conservative amino acid substitution thereof.

27. The immunomodulatory protein of embodiment 25 or embodiment 26, wherein the one or more amino acid substitutions comprise at least one of E74V, K77E, Y79F, L82H, L82P, R84G, R84L, R84Q, D85V, or C86Y.

28. The immunomodulatory protein of any of embodiments 25-27, wherein the one or more amino acid substitutions are D85E/K98T, I87L/K98T, L82P/I87L, G76S/P97S, K77E/R84L/F103Y, Y79F/Q99E, L83S/F103S, K77E/R84Q, K77E/A101D, K77E/F78Y/Y102D, Q75E/R84Q,

Q75R/R84G/I92V, K77E/A101D/Y102D, R84Q/S88N/A101D, R84Q/F103V, K77E/Q95R/A101D or I87M/A101D.

29. The immunomodulatory protein of any of embodiments 25-28, wherein the one or more amino acid substitutiosn are K77E/F78Y/Y102D.

30. The immunomodulatory protein of any of embodiments 25-28, wherein the one or more amino acid substitutions are Q75E/R84Q.

31. The immunomodulatory protein of any of embodiments 25-29, wherein the variant TACI polypeptide is set forth in SEQ ID NO: 541.

32. The immunomodulatory protein of any of embodiments 25-28 and 30, wherein the variant TACI polypeptide is set forth in SEQ ID NO:542.

33. The immunomodulatory protein of embodiment 14, wherein the TACI polypeptide is a variant TACI polypeptide that comprises one or more amino acid substitutions in the extracellular domain (ECD) of a reference TACI polypeptide or a specific binding fragment thereof at positions selected from among 40, 59, 60, 61, 74, 75, 76, 77, 78, 79, 82, 83, 84, 85, 86, 87, 88, 92, 95, 97, 98, 99, 101, 102 and 103, corresponding to numbering of positions set forth in SEQ ID NO:709.

34. The immunomodulatory protein of embodiment 33, wherein the reference TACI polypeptide is a truncated polypeptide consisting of the extracellular domain of TACI or a specific binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

35. The immunomodulatory protein of any of embodiments 33 and 34, wherein the reference TACI polypeptide comprises (i) the sequence of amino acids set forth in SEQ ID NO:709, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:709; or (iii) a portion of (i) or (ii) comprising one or both of a CRD1 domain and CRD2 domain that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

36. The immunomodulatory protein of any of embodiments 33-35, wherein the reference TACI polypeptide lacks an N-terminal methionine.

37. The immunomodulatory protein of any of embodiments 33-36, wherein the reference TACI polypeptide comprises the CRD1 domain and the CRD2 domain.

38. The immunomodulatory protein of any of embodiments 33-37, wherein the reference TACI polypeptide comprises the sequence set forth in SEQ ID NO:516.

39. The immunomodulatory protein of any of embodiments 33-37, wherein the reference TACI polypeptide consists of the sequence set forth in SEQ ID NO:516.

40. The immunomodulatory protein of any of embodiments 33-36, wherein the reference TACI polypeptide consists essentially of the CRD2 domain.

41. The immunomodulatory protein of any of embodiments 33-36 and 40, wherein the reference TACI polypeptide comprises the sequence set forth in SEQ ID NO:528.

42. The immunomodulatory protein of any of embodiments 33-36 and 40, wherein the reference TACI polypeptide consists of the sequence set forth in SEQ ID NO:528.

43. The immunomodulatory protein of any of embodiments 33-42, wherein the one or more amino acid substitutions are selected from W40R, Q59R, R60G, T61P E74V, Q75E, Q75R, G76S, K77E, F78Y, Y79F, L82H, L82P, L83S, R84G, R84L, R84Q, D85E, D85V, C86Y, I87L, I87M, S88N, I92V, Q95R, P97S, K98T, Q99E, A101D, Y102D, F103S, F103V, F103Y, or a conservative amino acid substitution thereof.

44. The immunomodulatory protein of any of embodiments 33-43, wherein the one or more amino acid substitutions comprise at least one of E74V, K77E, Y79F, L82H, L82P, R84G, R84L, R84Q, D85V or C86Y.

45. The immunomodulatory protein of any of embodiments 33-44, wherein the one or more amino acid substitution comprise at least the amino acid substitution K77E.

46. The immunomodulatory protein of any of embodiments 33-44, wherein the one or more amino acid substitution comprise at least the amino acid substitution R84G.

47. The immunomodulatory protein of any of embodiments 33-44, wherein the one or more amino acid substitution comprise at least the amino acid substitution R84Q.

48. The immunomodulatory protein of any of embodiments 33-47, wherein the one or more amino acid substitutions are D85E/K98T, I87L/K98T, R60G/Q75E/L82P, R60G/C86Y, W40R/L82P/F103Y, W40R/Q59R/T61P/K98T, L82P/I87L, G76S/P97S, K77E/R84L/F103Y, Y79F/Q99E, L83S/F103S, K77E/R84Q, K77E/A101D, K77E/F78Y/Y102D, Q75E/R84Q, Q75R/R84G/I92V, K77E/A101D/Y102D, R84Q/S88N/A101D, R84Q/F103V, K77E/Q95R/A101D or I87M/A101D.

49. The immunomodulatory protein of any of embodiments 33-44, 45 and 48, wherein the one or more amino acid substitutions are K77E/F78Y/Y102D.

50 The immunomodulatory protein of any of embodiments 33-44, 47 and 48, wherein the one or more amino acid substitutions are Q75E/R84Q.

51. The immunomodulatory protein of any of embodiments 33-50, wherein the variant TACI polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the reference TACI polypeptide.

52. The immunomodulatory protein of embodiment 24 or embodiment 51, wherein the variant TACI polypeptide has increased binding affinity to APRIL.

53. The immunomodulatory protein of embodiment 24 or embodiment 51, wherein the variant TACI polypeptide has increased binding affinity to BAFF.

54. The immunomodulatory protein of embodiment 24 or embodiment 51, wherein the variant TACI polypeptide has increased binding affinity to APRIL and BAFF.

55. The immunomodulatory protein of any of embodiments 24, and 51-54, wherein the increased binding affinity for BAFF or APRIL is independently increased more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold or 60-fold.

56. The immunomodulatory protein of any of embodiments 15-16, 17-21 and 24-55, wherein:

the variant TACI polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 517-527, 536, 537, 682-701; or

the variant TACI polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 529-535, 538-550, 673-681.

57. The immunomodulatory protein of any of embodiments 15-16, 17-21 and 24-55, wherein:

the variant TACI polypeptide consists or consists essentially of the sequence set forth in any one of SEQ ID NOS: 517-527, 536, 537, 682-701; or

the variant TACI polypeptide consists or consists essentially of the sequence set forth in any one of SEQ ID NOS: 529-535, 538-550, 673-681.

58. The immunomodulatory protein of any of embodiments 15-16, 17-21, 24-55 and 57, wherein the variant TACI polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 541.

59. The immunomodulatory protein of any of embodiments 15-16, 17-21, 24-55 and 57, wherein the variant TACI polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:542.

60. The immunomodulatory protein of any of embodiments 15-16, 17-21, 24-55 and 57, wherein the variant TACI polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:688.

61. The immunomodulatory protein of any of embodiments 15-16, 17-21, 24-55 and 57, wherein the variant TACI polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:535.

62. The immunomodulatory protein of any of embodiments 15-16, 17-21 and 24-61, comprising a heterologous moiety that is linked to the at least one TACI polypeptide.

63. The immunomodulatory protein of embodiment 62, wherein the heterologous moiety is a half-life extending moiety, a multimerization domain, a targeting moiety that binds to a molecule on the surface of a cell, or a detectable label.

64. The immunomodulatory protein of embodiment 63, wherein the half-life extending moiety comprises a multimerization domain, albumin, an albumin-binding polypeptide, Pro/Ala/Ser

(PAS), a C-terminal peptide (CTP) of the beta subunit of human chorionic gonadotropin, polyethylene glycol (PEG), long unstructured hydrophilic sequences of amino acids (XTEN), hydroxyethyl starch (HES), an albumin-binding small molecule, or a combination thereof.

65. The immunomodulatory protein of embodiment 17, wherein the BCMA polypeptide consists of the sequence set forth in SEQ ID NO:356.

66. The immunomodulatory protein of embodiment 17, wherein the BCMA polypeptide is a variant BCMA polypeptide comprising one or more amino acid substitutions in the extracellular domain (ECD) of a reference BCMA polypeptide or a specific binding fragment at positions selected from among 9, 10, 11, 14, 16, 19, 20, 22, 25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 and 48, corresponding to numbering set forth in SEQ ID NO:710.

67. An immunomodulatory protein comprising a variant BCMA polypeptide, wherein the variant BCMA polypeptide comprises one or more amino acid substitutions in the extracellular domain (ECD) of a reference BCMA polypeptide or a specific binding fragment thereof at positions selected from among 9, 10, 11, 14, 16, 19, 20, 22, 25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 and 48, corresponding to numbering of positions set forth in SEQ ID NO:710.

68. The immunomodulatory protein of embodiment 66 or embodiment 67, wherein the reference BCMA polypeptide is a polypeptide consisting of the extracellular domain of BCMA or a specific binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

69. The immunomodulatory protein of any of embodiments 66-68, wherein the reference BCMA polypeptide comprises (i) the sequence of amino acids set forth in SEQ ID NO:710, (ii) a sequence of amino acids that has at least 95% 37a.equence identity to SEQ ID NO:710; or (iii) a portion of (i) or (ii) comprising the CRD.

70. The immunomodulatory protein of any of embodiments 66-69, wherein the reference BCMA lacks an N-terminal methionine.

71. The immunomodulatory protein of any of embodiments 66-70, wherein the reference BCMA polypeptide comprises (i) the sequence of amino acids set forth in SEQ ID NO:356, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:356; or (iii) a portion of (i) or (ii) comprising the CRD.

72. The immunomodulatory protein of any of embodiments 66-71, wherein the reference BCMA polypeptide comprises the sequence set forth in SEQ ID NO:356.

73. The immunomodulatory protein of any of embodiments 66-71, wherein the reference BCMA polypeptide consists of the sequence set forth in SEQ ID NO:356.

74. The immunomodulatory protein of any of embodiments 66-73, wherein the one or more amino acid substitutions are selected from S9G, S9N, S9Y, Q10E, Q10P, N11D, N11S, F14Y, S16A, H19A, H19C, H19D, H19E, H19F, H19G, H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R,

H19S, H19T, H19V, H19W, H19Y, A20T, I22L, I22V, Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y, R27H, R27L, S29P, S30G, S30Y, N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y, T32I, T32S, L35A, L35M, L35P, L35S, L35V, L35Y, T36A, T36G, T36N, T36M, T36S, T36V, R39L, R39Q, A43E, A43S, V45A, V45D, V45I, T46A, T46I, N47D, N47Y, S48G, or a conservative amino acid substitution thereof.

75. The immunomodulatory protein of any of embodiments 66-74, wherein the one or more amino acid substitutions comprise at least one substitution at position 19, optionally wherein the at least one substitution is selected from H19A, H19C, H19D, H19E, H19F, H19G, H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R, H19S, H19T, H19V, H19W, H19Y.

76. The immunomodulatory protein of any of embodiments 66-75, wherein the one or more amino acid substitution comprise at least the amino acid substitution H19L.

77. The immunomodulatory protein of any of embodiments 66-75, wherein the one or more amino acid substitution comprise at least the amino acid substitution H19K.

78. The immunomodulatory protein of any of embodiments 66-75, wherein the one or more amino acid substitution comprise at least the amino acid substitution H19R.

79. The immunomodulatory protein of any of embodiments 66-75, wherein the one or more amino acid substitution comprise at least the amino acid substitution H19Y.

80. The immunomodulatory protein of any of embodiments 66-79, wherein the one or more amino acid substitutions comprise at least one substitution at position 25, optionally wherein the at least one substitution is selected from Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y.

81. The immunomodulatory protein of any of embodiments 66-80, wherein the one or more amino acid substitutions comprise at least one substitution at position 31, optionally wherein the at least one substitution is selected from N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y.

82. The immunomodulatory protein of any of embodiments 66-81, wherein the one or more amino acid substitutions comprise at least one substitution at position 35, optionally wherein the at least one substitution is selected from L35A, L35M, L35P, L35S, L35V, L35Y.

83. The immunomodulatory protein of any of embodiments 66-82, wherein the one or more amino acid substitutions comprise at least one substitution at position 36, optionally wherein the at least one substitution is selected from T36A, T36G, T36N, T36M, T36S, T36V.

84. The immunomodulatory protein of any of embodiments 66-83, wherein the one or more amino acid substitutions are H19Y/S30G; H19Y/V45A; F14Y/H19Y; H19Y/V45D; H19Y/A43E; H19Y/T36A; H19Y/I22V; N11D/H19Y; H19Y/T36M; N11S/H19Y; H19Y/L35P/T46A; H19Y/N47D; S9D/H19Y; H19Y/S30G/V45D; H19Y/R39Q; H19Y/L35P; S9D/H19Y/R27H; Q10P/H19Y/Q25H; H19Y/R39L/N47D; N11D/H19Y/N47D; H19Y/T32S; N11S/H19Y/S29P; H19Y/R39Q/N47D;

S16A/H19Y/R39Q; S9N/H19Y/N31K/T46I; H19Y/R27L/N31Y/T32S/T36A; N11S/H19Y/T46A; H19Y/T32I; S9G/H19Y/T36S/A43S; H19Y/S48G; S9N/H19Y/I22V/N31D; S9N/H19Y/Q25K/N31D; S9G/H19Y/T32S; H19Y/T36A/N47Y; H19Y/V45A/T46I; H19Y/Q25K/N31D; H19Y/Q25H/R39Q/V45D; H19Y/T32S/N47D; Q10E/H19Y/A20T/T36S; H19Y/T32S/V45I; H19F/Q25E/N31L/L35Y/T36S; H19F/Q25F/N31S/T36S; H19I/Q25F/N31S/T36V; H19F/Q25V/N31M/T36S; H19Y/Q25Y/N31L/L35Y/T36S; H19F/Q25I/N31M/L35A/T36S; H19I/Q25L/N31L/L35Y/T36S; H19F/Q25L/N31G/L35P/T36A; H19Y/I22L/N31G; H19F/I22V/Q25M/N31P/T36M; H19Y/N31L/L35Y/T36S; H19L/S30G/N31H/L35A; H19L/Q25S/N31V/L35S/T36V; H19L/Q25S/S30Y/N31G/L35M/T36V; H19F/Q25F/N31L/L35Y/T36S; H19F/Q25F/N31S/T36G; H19F/I22V/Q25S/N31V/L35S/T36V; H19F/Q25G/N31S/L35V/T36N; H19L/Q25H/N31D/L35S; or H19F/Q25F/N31S/L35Y/T36S.

85. The immunomodulatory protein of any of embodiments 66-75, 79, and 84, wherein the one or more amino acid substitutions comprise S16A/H19Y/R39Q.

86. The immunomodulatory protein of any of embodiments 66-85, wherein the variant BCMA polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the reference TACI polypeptide.

87. The immunomodulatory protein of embodiment 86, wherein the variant BCMA polypeptide has increased binding affinity to APRIL.

88. The immunomodulatory protein of embodiment 86, wherein the variant BCMA polypeptide has increased binding affinity to BAFF.

89. The immunomodulatory protein of embodiment 86, wherein the variant BCMA polypeptide has increased binding affinity to APRIL and BAFF.

90. The immunomodulatory protein of any of embodiments 86-89, wherein the increased binding affinity for BAFF or APRIL is independently increased more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold or 60-fold.

91. The immunomodulatory protein of any of embodiments 66-90, wherein the variant BCMA polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 357-435.

92. The immunomodulatory protein of any of embodiments 66-90, wherein the variant BCMA polypeptide consists or consists essentially of the sequence set forth in any one of SEQ ID NOS: 357-435.

93. The immunomodulatory protein of any of embodiments 66-90, wherein the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 381.

94. The immunomodulatory protein of any of embodiments 66-90, wherein the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:411.

95. The immunomodulatory protein of any of embodiments 66-90, wherein the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:405.

96. The immunomodulatory protein of any of embodiments 66-90, wherein the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:406.

97. The immunomodulatory protein of any of embodiments 17 and 66-96, comprising a heterologous moiety that is linked to the at least one BCMA polypeptide.

98. The immunomodulatory protein of embodiment 97, wherein the heterologous moiety is a half-life extending moiety, a multimerization domain, a targeting moiety that binds to a molecule on the surface of a cell, or a detectable label.

99. The immunomodulatory protein of embodiment 98, wherein the half-life extending moiety comprises a multimerization domain, albumin, an albumin-binding polypeptide, Pro/Ala/Ser (PAS), a C-terminal peptide (CTP) of the beta subunit of human chorionic gonadotropin, polyethylene glycol (PEG), long unstructured hydrophilic sequences of amino acids (XTEN), hydroxyethyl starch (HES), an albumin-binding small molecule, or a combination thereof.

100.The immunomodulatory protein of any of embodiments 17 and 66-99, comprising an Fc region of an immunoglobulin that is linked to the at least one BCMA polypeptide.

101.The immunomodulatory protein of any of embodiments 1-17, 18-20, 21-23, 24-33, 39-66 and 68-100, wherein the at least one TIM comprises only one TIM.

102. The immunomodulatory protein of any of embodiments 1-17, 18-20, 21-23, 24-33, 39-66 and 68-100, wherein the at least one TIM comprises 2, 3, 4, or 5 TIMs, optionally wherein each TIM is the same.

103. The immunomodulatory protein of embodiment 102, wherein each TIM is linked directly or indirectly via a linker, optionally wherein the linker is a peptide linker.

104. The immunomodulatory protein of any of embodiments 1-17, 18-20, 21-23, 24-33, 39-66 and 68-103, wherein the at least one BIM comprises only one BIM.

105. The immunomodulatory protein of any of embodiments 1-17, 18-20, 21-23, 24-33, 39-66 and 68-103, wherein the at least one BIM comprises 2, 3, 4, or 5 BIMs, optionally wherein each BIM is the same.

106. The immunomodulatory protein of embodiment 105, wherein each BIM is linked directly or indirectly via a linker, optionally wherein the linker is a peptide linker

107. The immunomodulatory protein of embodiment 103 or embodiment 106, wherein the linker is a peptide linker and the peptide linker is selected from GSGGS (SEQ ID NO: 592), GGGGS (G4S; SEQ ID NO: 593), GSGGGGS (SEQ ID NO: 590), GGGGSGGGGS (2xGGGGS; SEQ ID NO: 594), GGGGSGGGGSGGGGS (3xGGGGS; SEQ ID NO: 595), GGGGSGGGGSGGGGSGGGGS

(4xGGGGS, SEQ ID NO:600), GGGGSGGGGSGGGGSGGGGSGGGGS (5XGGGGS, SEQ ID NO: 671), GGGGSSA (SEQ ID NO: 596) or combinations thereof.

108. The immunomodulatory protein of any of embodiments 1-17, 18-20, 21-23, 24-33, 39-66 and 68-107wherein the at least one TIM and the at least one BIM are linked directly or indirectly via a linker, optionally wherein the linker comprises a peptide linker and/or a multimerization moiety.

109. The immunomodulatory protein of embodiment 108, wherein the linker comprises a peptide linker and the peptide linker is selected from GSGGS (SEQ ID NO: 592), GGGGS (G4S; SEQ ID NO: 593), GSGGGGS (SEQ ID NO: 590), GGGGSGGGGS (2xGGGGS; SEQ ID NO: 594), GGGGSGGGGSGGGGS (3xGGGGS; SEQ ID NO: 595), GGGGSGGGGSGGGGSGGGGS (4xGGGGS, SEQ ID NO:600), GGGGSGGGGSGGGGSGGGGSGGGGS (5XGGGGS, SEQ ID NO: 671), GGGGSSA (SEQ ID NO: 596) or combinations thereof.

110. The immunomodulatory protein of embodiment 108, wherein the linker comprises a peptide linker and the peptide linker is selected from SEQ ID NO: 711 (1xEAAAK), SEQ ID NO: 712 (2xEAAAK), SEQ ID NO: 713 (3xEAAAK), SEQ ID NO: 714 (4xEAAAK), SEQ ID NO: 715 (5xEAAAK), SEQ ID NO: 665 (6xEAAAK) .

111. The immunomodulatory protein of any of embodiments 1-17, 18-20, 21-23, 24-33, 34-66 and 68-110, wherein the immunomodulatory protein is a monomer and/or comprises a single polypeptide chain.

112. The immunomodulatory protein of embodiment 111, wherein the at least one TIM is amino-terminal to the at least one BIM in the polypeptide.

113. The immunomodulatory protein of embodiment 111, wherein the at least one TIM is carboxy-terminal to the at least one BIM in the polypeptide.

114. The immunomodulatory protein of any of embodiments 1-17, 18-20, 21-23, 24-33, 39-66 and 68-113, wherein the immunomodulatory protein further comprises a detectable label, optionally wherein the detectable label is a Flag tag, a His tag, or a myc tag.

115. The immunomodulatory protein of any of embodiments 1-17, 18-20, 21-23, 24-33, 39-64, and 101-114, wherein the immunomodulatory protein comprises the sequence of amino acids set forth in any of SEQ ID NOS: 618-623, or a sequence that exhibits at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto and retains activity.

116. The immunomodulatory protein of any of embodiments 18-20, 21-23, 24-33, 39-64, and 101-113, wherein the immunomodulatory protein comprises the sequence of amino acids set forth in any of SEQ ID NOS: 703-708, or a sequence that exhibits at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto and retains activity.

117. The immunomodulatory protein of embodiment 108, wherein the linker comprises a multimerization domain wherein the multimerization domain promotes dimerization, trimerization, tetramerization, or pentamerization.

118. The immunomodulatory protein of embodiment 108 or embodiment 117, wherein the multimerization domain is an immunoglobulin Fc region.

119. The immunomodulatory protein of any of embodiments 1-16, 18-20, 21-23, 24-33, 39-66 and 68-110117 and 118, wherein the immunomodulatory protein is a dimer.

120. The immunomodulatory protein of embodiment 100 and 118, wherein the immunoglobulin Fc region is a homodimeric Fc region.

121. The immunomodulatory protein of embodiment 100 and 118, wherein the immunoglobulin Fc region is a heterodimeric Fc region

122. The immunomodulatory protein of embodiment 1-16, 18-20, 21-23, 24-33, 39-66 and 68-110, and 117-120, wherein the immunomodulatory protein is a homodimer, wherein each polypeptide of the dimer is the same.

123. The immunomodulatory protein of embodiment 122, wherein each polypeptide comprises the at least one TIM and the at least one BIM and wherein the at least one TIM is amino-terminal to the at least one BIM in each polypeptide.

124. The immunomodulatory protein of embodiment 122, wherein each polypeptide comprises the at least one TIM and the at least one BIM and wherein the at least one TIM is carboxy-terminal to the at least one BIM in each polypeptide.

125. The immunomodulatory protein of any of embodiments 100, embodiments 118-120 and 122-124, wherein the immunoglobulin Fc is an IgG1 Fc domain, or is a variant Fc that exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, optionally as compared to a wild-type IgG1 Fc domain.

126. The immunomodulatory protein of any of embodiments 100, embodiments 118-120 and 122-125, wherein the immunoglobulin Fc is an IgG1 Fc domain and the Fc comprises the amino acid sequence set forth in SEQ ID NO: 597.

127. The immunomodulatory protein of any of embodiments 100, embodiments 118-120 and 122-125, wherein the immunoglobulin Fc is a variant IgG1 Fc domain comprising one or more amino acid substitutions selected from L234A, L234V, L235A, L235E, G237A, S267K, R292C, N297G, and V302C, by EU numbering.

128. The immunomodulatory protein of embodiment 127, wherein the immunoglobulin Fc region contains the amino acid substitutions L234A, L235E an G237A by EU numbering or the amino acid substitutions R292C, N297G and V302C by EU numbering.

129. The immunomodulatory protein of any of embodiments 100, embodiments 118-120 and 122-125, embodiment 127 and embodiment 128, wherein the Fc is a variant Fc comprising the amino acid sequence set forth in SEQ ID NO:589.

130. The immunomodulatory protein of any of embodiments 1-16, 18-20, 21-23, 24-33, 39-65 and 68-110, and 117-128,, wherein the immunomodulatory protein comprises the sequence of amino acids set forth in any of SEQ ID NOS: 610-617, 624-627, 637, 638, 643, 644, 648, 653 and 654, or a sequence that exhibits at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto and retains activity.

131. The immunomodulatory protein of any of embodiments 1-13, 17, 65, 66, 68-110, and 117-128, wherein the immunomodulatory protein comprises the sequence of amino acids set forth in any of SEQ ID NOS: 601-609, 631-636, 645-647, 649-652, 655-659, or a sequence that exhibits at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto and retains activity.

132. The immunomodulatory protein of embodiment 1-16, 18-20, 21-23, 24-33, 39-66, 68-110, 117-119 and 121wherein the immunomodulatory protein is a heterodimer, wherein each polypeptide of the dimer is linked to an immunoglobulin Fc domain individually comprising one or more amino acid modifications in a wild-type Fc domain to effect heterodimer formation between the polypeptides.

133. The immunomodulatory protein of embodiment 132, wherein the wild-type immunoglobulin Fc is an IgG1 Fc domain.

134. The immunomodulatory protein of embodiment 132 or embodiment 133, wherein the one more amino acid modifications are selected from a knob-into-hole modification and a charge mutation to reduce or prevent self-association due to charge repulsion.

135. The immunomodulatory protein of any of embodiments 132-134, further comprising one or more amino acid substitutions to reduced binding affinity to an Fc receptor and/or reduced effector function, optionally as compared to a wild-type IgG1 Fc domain.

136. The immunomodulatory protein of embodiment 135, wherein the one or more amino acid substitutions are selected from L234A, L234V, L235A, L235E, G237A, S267K, R292C, N297G, and V302C, by EU numbering.

137. The immunomodulatory protein of embodiment 135 or embodiment 136, wherein the immunoglobulin Fc region contains the amino acid substitutions L234A, L235E an G237A by EU numbering or the amino acid substitutions R292C, N297G and V302C by EU numbering.

138. The immunomodulatory protein of any of embodiments 132-137, wherein the heterodimer comprises a first polypeptide comprising the sequence of amino acids set forth in SEQ ID NO: 662 or 663 and a second polypeptide comprising the sequence of amino acids set forth in SEQ ID NO:660.

139. The immunomodulatory protein of any of embodiments 1-138, wherein:

the immunomodulatory protein blocks binding of APRIL, BAFF, or an APRIL/BAFF heterotrimer to BCMA or TACI; and/or

the immunomodulatory protein reduces the levels of circulating APRIL, BAFF, or an APRIL/BAFF in the blood following administration to a subject.

140. The immunomodulatory protein of any of embodiments 1-138, wherein the immunomodulatory protein reduces or inhibits B cell maturation, differentiation and/or proliferation.

141. The immunomodulatory protein of any of embodiments 1-17, 18-20, 21-34, 34-6668-134, wherein:

the immunomodulatory protein blocks binding of CD80 or CD86 to a costimulatory receptor, optionally wherein the costimulatory receptor is CD28; and/or

the immunomodulatory protein reduces or inhibits T cell costimulation.

142. A nucleic acid molecule(s) encoding the immunomodulatory protein of any of embodiments 1-141.

143. The nucleic acid molecule of embodiment 142 that is a synthetic nucleic acid.

144. The nucleic acid molecule of embodiment 142 or embodiment 143 that is a cDNA.

145. A vector, comprising the nucleic acid molecule of any of embodiments 142-144.

146. The vector of embodiment 145 that is an expression vector.

147. The vector of embodiment 145 or embodiment 146, wherein the vector is a mammalian expression vector or a viral vector.

148. A cell, comprising the nucleic acid of any of embodiments 142-144 or the vector of any of any of embodiments 145-147.

149. The cell of embodiment 148 that is a mammalian cell.

150. The cell of embodiment 148 or embodiment 149 that is a human cell.

151. A method of producing an immunomodulatory protein, comprising introducing the nucleic acid molecule of any of embodiments 142-144 or vector of any of embodiments 145-147 into a host cell under conditions to express the protein in the cell.

152. The method of embodiment 151, further comprising isolating or purifying the immunomodulatory protein from the cell.

153. An immunomodulatory protein produced by the method of embodiment 151 or embodiment 152.

154. A pharmaceutical composition, comprising the immunomodulatory protein of any of embodiments 1-141 and 153.

155. A variant BCMA-Fc fusion protein comprising a variant BCMA polypeptide, an Fc region, and a linker between the BCMA polypeptide and Fc region, wherein the variant BCMA

polypeptide comprises one or more amino acid substitutions in the extracellular domain (ECD) of an unmodified BCMA polypeptide or a specific binding fragment thereof corresponding to positions selected from among 9, 10, 11, 14, 16, 19, 20, 22, 25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 and 48 with reference to positions set forth in SEQ ID NO:710.

156. The variant BCMA-Fc fusion protein of embodiment 155, wherein the reference BCMA polypeptide is a polypeptide consisting of the extracellular domain of BCMA or a specific binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

157. The variant BCMA-Fc fusion protein of embodiment 155 or embodiment 156, wherein the reference BCMA polypeptide comprises (i) the sequence of amino acids set forth in SEQ ID NO:710, (ii) a sequence of amino acids that has at least 95% 37a.equence identity to SEQ ID NO:710; or (iii) a portion of (i) or (ii) comprising the CRD.

158. The variant BCMA-Fc fusion protein of any of embodiments 155-157, wherein the reference BCMA lacks an N-terminal methionine.

159. The variant BCMA-Fc fusion protein of any of embodiments 155-158, wherein the reference BCMA polypeptide comprises (i) the sequence of amino acids set forth in SEQ ID NO:356, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:356; or (iii) a portion of (i) or (ii) comprising the CRD.

160. The variant BCMA-Fc fusion protein of any of embodiments 155-159, wherein the reference BCMA polypeptide comprises the sequence set forth in SEQ ID NO:356.

161. The variant BCMA-Fc fusion protein of any of embodiments 155-159, wherein the reference BCMA polypeptide consists of the sequence set forth in SEQ ID NO:356.

162. The variant BCMA-Fc fusion protein of any of embodiments 155-161, wherein the one or more amino acid substitutions are selected from S9G, S9N, S9Y, Q10E, Q10P, N11D, N11S, F14Y, S16A, H19A, H19C, H19D, H19E, H19F, H19G, H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R, H19S, H19T, H19V, H19W, H19Y, A20T, I22L, I22V, Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y, R27H, R27L, S29P, S30G, S30Y, N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y, T32I, T32S, L35A, L35M, L35P, L35S, L35V, L35Y, T36A, T36G, T36N, T36M, T36S, T36V, R39L, R39Q, A43E, A43S, V45A, V45D, V45I, T46A, T46I, N47D, N47Y, S48G, or a conservative amino acid substitution thereof.

163. The variant BCMA-Fc fusion protein of any of embodiments 155-162, wherein the one or more amino acid substitutions comprise at least one substitution at position 19, optionally wherein the at least one substitution is selected from H19A, H19C, H19D, H19E, H19F, H19G, H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R, H19S, H19T, H19V, H19W, H19Y.

164. The variant BCMA-Fc fusion protein of any of embodiments 155-163, wherein the one or more amino acid substitution comprise at least the amino acid substitution H19L.

165. The variant BCMA-Fc fusion protein of any of embodiments 155-163, wherein the one or more amino acid substitution comprise at least the amino acid substitution H19K.

166. The variant BCMA-Fc fusion protein of any of embodiments 155-163, wherein the one or more amino acid substitution comprise at least the amino acid substitution H19R.

167. The variant BCMA-Fc fusion protein of any of embodiments 155-163, wherein the one or more amino acid substitution comprise at least the amino acid substitution H19Y.

168. The variant BCMA-Fc fusion protein of any of embodiments 155-167, wherein the one or more amino acid substitutions comprise at least one substitution at position 25, optionally wherein the at least one substitution is selected from Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y.

169. The variant BCMA-Fc fusion protein of any of embodiments 155-168, wherein the one or more amino acid substitutions comprise at least one substitution at position 31, optionally wherein the at least one substitution is selected from N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y.

170. The variant BCMA-Fc fusion protein of any of embodiments 155-169, wherein the one or more amino acid substitutions comprise at least one substitution at position 35, optionally wherein the at least one substitution is selected from L35A, L35M, L35P, L35S, L35V, L35Y.

171. The variant BCMA-Fc fusion protein of any of embodiments 155-170, wherein the one or more amino acid substitutions comprise at least one substitution at position 36, optionally wherein the at least one substitution is selected from T36A, T36G, T36N, T36M, T36S, T36V.

172. The variant BCMA-Fc fusion protein of any of embodiments 155-171, wherein the one or more amino acid substitutions are H19Y/S30G; H19Y/V45A; F14Y/H19Y; H19Y/V45D; H19Y/A43E; H19Y/T36A; H19Y/I22V; N11D/H19Y; H19Y/T36M; N11S/H19Y; H19Y/L35P/T46A; H19Y/N47D; S9D/H19Y; H19Y/S30G/V45D; H19Y/R39Q; H19Y/L35P; S9D/H19Y/R27H; Q10P/H19Y/Q25H; H19Y/R39L/N47D; N11D/H19Y/N47D; H19Y/T32S; N11S/H19Y/S29P; H19Y/R39Q/N47D; S16A/H19Y/R39Q; S9N/H19Y/N31K/T46I; H19Y/R27L/N31Y/T32S/T36A; N11S/H19Y/T46A; H19Y/T32I; S9G/H19Y/T36S/A43S; H19Y/S48G; S9N/H19Y/I22V/N31D; S9N/H19Y/Q25K/N31D; S9G/H19Y/T32S; H19Y/T36A/N47Y; H19Y/V45A/T46I; H19Y/Q25K/N31D; H19Y/Q25H/R39Q/V45D; H19Y/T32S/N47D; Q10E/H19Y/A20T/T36S; H19Y/T32S/V45I; H19F/Q25E/N31L/L35Y/T36S; H19F/Q25F/N31S/T36S; H19I/Q25F/N31S/T36V; H19F/Q25V/N31M/T36S; H19Y/Q25Y/N31L/L35Y/T36S; H19F/Q25I/N31M/L35A/T36S; H19I/Q25L/N31L/L35Y/T36S; H19F/Q25L/N31G/L35P/T36A; H19Y/I22L/N31G; H19F/I22V/Q25M/N31P/T36M; H19Y/N31L/L35Y/T36S; H19L/S30G/N31H/L35A; H19L/Q25S/N31V/L35S/T36V; H19L/Q25S/S30Y/N31G/L35M/T36V; H19F/Q25F/N31L/L35Y/T36S;

H19F/Q25F/N31S/T36G; H19F/I22V/Q25S/N31V/L35S/T36V; H19F/Q25G/N31S/L35V/T36N; H19L/Q25H/N31D/L35S; or H19F/Q25F/N31S/L35Y/T36S.

173. The variant BCMA-Fc fusion protein of any of embodiments 155-163, 167, and 172, wherein the one or more amino acid substitutions comprise S16A/H19Y/R39Q.

174. The variant BCMA-Fc fusion protein of any of embodiments 155-173, wherein the variant BCMA polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the reference BCMA polypeptide.

175. The variant BCMA-Fc fusion protein of any of embodiments 155-174, wherein the variant BCMA polypeptide has increased binding affinity to APRIL.

176. The variant BCMA-Fc fusion protein of any of embodiments 155-174, wherein the variant BCMA polypeptide has increased binding affinity to BAFF.

177. The variant BCMA-Fc fusion protein of any of embodiments 155-174, wherein the variant BCMA polypeptide has increased binding affinity to APRIL and BAFF.

178. The variant BCMA-Fc fusion protein of any of embodiments 174-177, wherein the increased binding affinity for BAFF or APRIL is independently increased more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold or 60-fold.

179. The variant BCMA-Fc fusion protein of any of embodiments 155-178, wherein the variant BCMA polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 357-435.

180. The variant BCMA-Fc fusion protein of any of embodiments 155-178, wherein the variant BCMA polypeptide consists or consists essentially of the sequence set forth in any one of SEQ ID NOS: 357-435.

181. The variant BCMA-Fc fusion protein of any of embodiments 155-178, wherein the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO: 381.

182. The variant BCMA-Fc fusion protein of any of embodiments 155-178, wherein the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:411.

183. The variant BCMA-Fc fusion protein of any of embodiments 155-178, wherein the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:405.

184. The variant BCMA-Fc fusion protein of any of embodiments 155-178, wherein the variant BCMA polypeptide consists or consists essentially of the sequence set forth in SEQ ID NO:406.

185. The Fc fusion protein of any of embodiments 155-184, wherein the linker comprises a peptide linker and the peptide linker is selected from GSGGS (SEQ ID NO: 592), GGGGS (G4S; SEQ ID NO: 593), GSGGGGS (SEQ ID NO: 590), GGGGSGGGGS (2xGGGGS; SEQ ID NO: 594), GGGGSGGGGSGGGGS (3xGGGGS; SEQ ID NO: 595), GGGGSGGGGSGGGGSGGGGS

(4xGGGGS, SEQ ID NO:600), GGGGSGGGGSGGGGSGGGGSGGGGS (5XGGGGS, SEQ ID NO: 671), GGGGSSA (SEQ ID NO: 596) or combinations thereof.

186. The Fc fusion protein of any of embodiments 155-185 that is a dimer.

187. The Fc fusion protein of any of embodiments 155-186, wherein the immunoglobulin Fc region is a homodimeric Fc region.

188. The Fc fusion protein of any of embodiments155-187, wherein the immunoglobulin Fc is an IgG1 Fc domain, or is a variant Fc that exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, optionally as compared to a wild-type IgG1 Fc domain.

189. The Fc fusion protein of any of embodiments 155-188, wherein the immunoglobulin Fc is an IgG1 Fc domain and the Fc comprises the amino acid sequence set forth in SEQ ID NO: 597.

190. The Fc fusion protein of any of embodiments 155-188, wherein the immunoglobulin Fc is a variant IgG1 Fc domain comprising one or more amino acid substitutions selected from L234A, L234V, L235A, L235E, G237A, S267K, R292C, N297G, and V302C, by EU numbering.

191. The Fc fusion protein of embodiment 190, wherein the immunoglobulin Fc region contains the amino acid substitutions L234A, L235E an G237A by EU numbering or the amino acid substitutions R292C, N297G and V302C by EU numbering.

192. The Fc fusion protein of any of embodiments 155-191, wherein the immunoglobulin Fc is set forth in SEQ ID NO:586.

193. The Fc fusion protein of any of embodiments 155-192, wherein the Fc is a variant Fc comprising the amino acid sequence set forth in SEQ ID NO:589.

194. The Fc fusion protein of any of embodiments 155-193 that is a dimer.

195. The Fc fusion protein of any of embodiments 155-194 that is a homodimer.

196. The Fc fusion protein of any of embodiments 155-195, wherein the Fc fusion protein neutralizes APRIL and BAFF.

197. The Fc fusion protein of any of embodiments 155-196, wherein:

the IC50 for neutralizing APRIL is less than 100 pM, less than 50 pM, less than 40 pM, less than 30 pM, less than 20 pM, less than 10 pM, less than 5 pM or less than 1 pM, or is any value between any of the foregoing; and/or

the IC50 for neutralizing BAFF is less than 400 pM, less than 300 pM, less than 200 pM, less than 100 pM, less than 75 pM, less than 50 pM, less than 25 pm, or less than 10 pM, or is any value between any of the foregoing.

198. The Fc fusion protein of any of embodiments 155-196, wherein:

the Fc fusion protein blocks binding of APRIL, BAFF, or an APRIL/BAFF heterotrimer to BCMA or TACI; and/or

the Fc fusion protein reduces the levels of circulating APRIL, BAFF, or an APRIL/BAFF in the blood following administration to a subject.

199. The Fc fusion protein of any of embodiments 155-198, wherein the immunomodulatory protein reduces or inhibits B cell maturation, differentiation and/or proliferation.

200. A nucleic acid molecule(s) encoding the Fc fusion protein of any of embodiments 155-199.

201. The nucleic acid molecule of embodiment 200 that is a synthetic nucleic acid.

202. The nucleic acid molecule of embodiment 200 or embodiment 201 that is a cDNA.

203. A vector, comprising the nucleic acid molecule of any of embodiments 200-202.

204. The vector of embodiment 203 that is an expression vector.

205. The vector of embodiment 203 or embodiment 204, wherein the vector is a mammalian expression vector or a viral vector.

206. A cell, comprising the nucleic acid of any of embodiments 200-202 or the vector of any of any of embodiments 203-205.

207. The cell of embodiment 206 that is a mammalian cell.

208. The cell of embodiment 206 or embodiment 207 that is a human cell.

209. A method of producing an Fc fusion protein, comprising introducing the nucleic acid molecule of any of embodiments 200-202 or vector of any of embodiments 203-205 into a host cell under conditions to express the protein in the cell.

210. The method of embodiment 209, further comprising isolating or purifying the Fc fusion protein from the cell.

211. An Fc fusion protein produced by the method of embodiment 209 or embodiment 210.

212. A pharmaceutical composition, comprising the Fc fusion protein of any of embodiments 155-199 and211.

213. The pharmaceutical composition of embodiment 154 or embodiment 212, comprising a pharmaceutically acceptable excipient.

214. The pharmaceutical composition of any of embodiments 154, 212 and 213, wherein the pharmaceutical composition is sterile.

215. An article of manufacture comprising the pharmaceutical composition of any of embodiments 154 and 212-214 in a vial or container.

216. The article of manufacture of embodiment 215, wherein the vial or container is sealed.

217. A kit comprising the pharmaceutical composition of any of embodiments 154 and212-214, and instructions for use.

218. A kit comprising the article of manufacture of embodiment 215 or embodiment 216, and instructions for use.

219. A method of reducing an immune response in a subject, comprising administering the immunomodulatory protein of any of embodiments 1-141 or 153 to a subject in need thereof.

220. A method of reducing an immune response in a subject, comprising administering the Fc fusion protein of any of embodiments 155-199 and 211 to a subject in need thereof.

221. A method of reducing an immune response in a subject, comprising administering the pharmaceutical composition of any of embodiments 154 and 211-214 to a subject in need thereof.

222. The method of any of embodiments 219-221, wherein a B cell immune response is reduced in the subject, whereby B cell maturation, differentiation and/or proliferation is reduced or inhibited.

223. The method of any of embodiments 219-222, wherein circulating levels of APRIL, BAFF or an APRIL/BAFF heterotrimer are reduced in the subject.

224. A method of reducing circulating levels of APRIL, BAFF or an APRIL/BAFF heterotrimer in a subject comprising administering the pharmaceutical composition of any of embodiments 155 and 211-214 to the subject.

225. The method of embodiment 128 or embodiment221, wherein a T cell immune response is reduced in the subject, whereby T cell costimulation is reduced or inhibited.

226. The method of any of embodiments 219-225, wherein reducing the immune response treats a disease or condition in the subject.

227. A method of treating a disease or condition in a subject, comprising administering the immunomodulatory protein of any of embodiments 1-141 or 153 to a subject in need thereof.

228. A method of treating a disease or condition in a subject, comprising administering the Fc fusion protein of any of claims 155-199to a subject in need thereof.

229. A method of treating a disease or condition in a subject, comprising administering the pharmaceutical composition of any of embodiments 154 and 211-214 to a subject in need thereof.

230. The method of any of embodiments 224-227, wherein the disease or condition is an autoimmune disease, a B cell cancer, an antibody- mediated pathology, a renal disease, a graft rejection, graft versus host disease, or a viral infection.

231. The method of embodiment 230, wherein the disease or condition is an autoimmune disease selected from the group consisting of Systemic lupus erythematosus (SLE); Sjögren's syndrome, scleroderma, Multiple sclerosis, diabetes, polymyositis, primary biliary cirrhosis, IgA nephropathy, optic neuritis, amyloidosis, antiphospholipid antibody syndrome (APS), autoimmune polyglandular syndrome type II (APS II), autoimmune thyroid disease (AITD), Graves' disease, autoimmune adrenalitis and pemphigus vulgaris.

232. The method of embodiment 230, wherein the disease or condition is a B cell cancer and the cancer is myeloma.

## X. EXAMPLES

**[0609]** The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

### Example 1. Generation and Assessment of variant CTLA-4 extracellular domain polypeptides as a T cell Inhibitory Molecule (TIM)

**[0610]** This examples describes exemplary CTLA-4 polypeptide T cell inhibitory molecules (TIMs) that are employed as part of a provided multi-domain immunomodulatory protein with a B cell inhibitory molecule (BIM), including methods for engineering and identifying affinity-modified (variant) CTLA-4 polypeptides that bind (e.g. increased compared to wild-type) to ligands of a T cell stimulatory receptor.

**[0611]** Mutant DNA constructs of human CTLA-4 IgSF domains were generated for translation and expression on the surface of yeast as yeast display libraries.

**[0612]** Libraries containing random substitutions of amino acids were constructed to identify variants of the extracellular domain (ECD) of CTLA-4 containing an immunoglobulin superfamily (IgSF) domain (CTLA-4 vIgD) based on a wild-type human CTLA-4 sequence set forth in SEQ ID NO: 1 as follows:.

KAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNEL TFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPD SD (SEQ ID NO:1)

**[0613]** DNA encoding the wild-type CTLA-4 ECD was cloned between the BamHI and KpnI sites of the modified yeast display vector pBYDS03 (Life Technologies, USA). Mutations were introduced via error prone PCR utilizing the Genemorph II Kit (Agilent, USA) supplemented with $MnCl_2$ and using ECD-specific oligonucleotides which overlapped by 40 bp with pBYDS03 cloning vector beyond and including the BamHI and KpnI cloning sites. Mutagenized DNA PCR product was purified via agarose gel electrophoresis then further amplified using 100 ng of mutagenized PCR product with OneTaq 2x PCR Master Mix (New England Biolabs, USA). The products from the second PCR were purified via agarose gel electrophoresis and PCR-Gel purification (Qiagen, Germany) and resuspended in sterile deionized water. A total of 12 μg of PCR product was generated for each subsequent yeast electroporation.

**[0614]** To prepare for library insertion, pBYDS03 vector was digested with BamHI and KpnI restriction enzymes (New England Biolabs, USA) and the large vector fragment was gel-purified and dissolved in sterile, deionized water. Electroporation-ready DNA for the next step was generated by

mixing 12 μg of library DNA insert with 4 μg of linearized vector in a total volume of 50 μL deionized and sterile water.

**[0615]** The CTLA-4 DNA libraries were introduced into yeast using electroporation. Briefly, electroporation-competent cells of yeast strain BJ5464 (ATCC.org; ATCC number 208288) were prepared and electroporated on a Gene Pulser II (Biorad, USA) with the electroporation-ready DNA from the steps above essentially as described (Colby, D.W. et al. 2004 Methods Enzymology 388, 348-358). The only exception is that transformed cells were grown in non-inducing minimal selective SCD-Leu medium to accommodate the LEU2 selectable marker carried by modified plasmid pBYDS03. One liter of SCD-Leu media was generated with 14.7 grams sodium citrate, 4.29 grams citric acid monohydrate, 20 grams dextrose, 6.7 grams yeast nitrogen base, and 1.6 grams yeast synthetic drop-out media supplement without leucine. The Medium was filter sterilized before use using a 0.22 μm vacuum filter device.

**[0616]** Library size was determined by plating serial dilutions of freshly recovered cells on SCD-Leu agar plates and then extrapolating library size from the number of single colonies from plating that generated at least 50 colonies per plate. In general, library sizes ranged from $1.0 \times 10^8$ to $1 \times 10^9$ transformants based on this dilution assay. The remainder of the electroporated culture was grown to saturation in SCD-Leu and cells from this culture were subcultured (e.g., 1/100) into fresh SCD-Leu once more to minimize the fraction of untransformed cells, and grown overnight. To maintain library diversity, this subculturing step was carried out using an inoculum that contained at least 10 times more cells than the calculated library size. Cells from the second saturated culture were resuspended in fresh medium containing sterile 25% (weight/volume) glycerol to a density of $1 \times 10^{10}$/mL and frozen and stored at -80 ºC (frozen library stock).

**[0617]** Yeast, expressing affinity modified variants of CTLA-4 IgD were selected against ICOSL and/or CD86. A number of cells equal to at least 10 times the estimated library size were thawed from individual library stocks, suspended to $1.0 \times 10^6$ cells/mL in non-inducing SCD-Leu medium, and grown overnight. The next day, a number of cells equal to 10 times the library size were centrifuged at 2000 RPM for two minutes and resuspended to $5.0 \times 10^6$ cells/mL in inducing SCDG-Leu medium. One liter of the SCDG-Leu induction media consisted of 5.4 grams $Na_2HPO_4$, 8.56 grams of $NaH_2PO_4 \bullet H_20$, 20 grams galactose, 2.0 grams dextrose, 6.7 grams yeast nitrogen base, and 1.6 grams of yeast synthetic drop out media supplement without leucine dissolved in water and sterilized through a 0.22 μm membrane filter device. The culture was grown in induction medium for 1 day at room temperature to induce expression of library proteins on the yeast cell surface.

**[0618]** The induced yeast library underwent 4 cycles of bead sorts using magnetic beads loaded alternately with ICOSL or CD86 to reduce non-binders and enrich for variant CTLA-4 molecules with the ability to bind ICOSL or CD86. After each cycle of selection, yeast retained through binding to

magnetic beads were amplified through growth in SCD media followed by overnight induction in SCDG media. The preliminary selection was followed by two rounds of fluorescence activated cell sorting (FACS) using ICOSL-Fc in round 1 and CD86-Fc in round 2 to enrich the fraction of yeast cells that displays improved binders. Magnetic bead enrichment and selections by flow cytometry were carried out essentially as described in Miller et al., Current Protocols in Cytometry 4.7.1-4.7.30, July 2008.

**[0619]** This selection process utilized the following reagents and instruments: human rICOSL.Fc (i.e., recombinant ICOSL-Fc fusion protein) and human rCD86.Fc target ligand proteins were purchased from R & D Systems, USA. Magnetic Protein A beads were obtained from New England Biolabs, USA. For two-color, flow cytometric sorting, a Bio-Rad S3e sorter was used. CTLA-4 display levels were monitored with an anti-hemagglutinin antibody labeled with Alexafluor 488 (Life Technologies, USA). Ligand binding of Fc fusion proteins, rICOSL.Fc or rCD86.Fc, were detected with PE-conjugated human Ig specific goat Fab (Jackson ImmunoResearch, USA). Doublet yeast were gated out using forward scatter (FSC) / side scatter (SSC) parameters, and sort gates were based upon higher ligand binding detected in FL2 that possessed more limited tag expression binding in FL1.

**[0620]** Yeast outputs from the flow cytometric sorts were assayed for higher specific binding affinity. Sort output yeast were expanded and re-induced to express the particular IgSF affinity modified domain variants they encode. This population was then compared to the parental, wild-type yeast strain, or other selected outputs, such as the bead output yeast population, by flow cytometry.

**[0621]** After the second round of FACS the output was serially diluted and plated onto SCD-agar such that single clones could be isolated. Two hundred and eighty-eight colonies were picked into round bottom microtiter plates containing 150 µL SCD media supplemented with kanamycin, penicillin and streptomycin. Plates were incubated at 30 °C with shaking. After 4h of growth, 80 µL were transferred to wells of a new plate, cells were spun down, SCD removed, 200 µL of SCDG induction media supplemented with antibiotics were added to each well followed by overnight incubation at room temperature with shaking. FACS analysis was used to independently assess binding of each clone to rICOSL-Fc, rCD86-Fc and anti-HA Mab as a control for expression. Control wells of yeast bearing wildtype CTLA-4 were run on each plate. 16 clones were selected to be reformatted into Fc fusion constructs and sequenced as described below.

**[0622]** Sequence analysis of the 16 yeast clones revealed a single dominant combination of mutations (L12P/A26T/L63P/L98Q/Y105L; SEQ ID NO: 3). In order to generate additional clonal diversity and determine the minimal mutations required for enhanced binding, the mutations in this clone were partially shuffled with wildtype sequence. Briefly, three pairs of PCR primers were designed that divided the ECD coding region into thirds. The PCR primers maintained 20 bp overlapping sequence with adjacent PCR product in order to facilitate subsequent Gibson Assembly cloning. Three PCR products were generated from both wildtype $A_1$, $B_1$, $C_1$) and mutant template ($A_2$, $B_2$, $C_2$). Combinations

of 3 PCR products, e.g. $A_2$,$B_1$,$C_1$; $A_2$,$B_2$,$C_1$ etc., were mixed with a modified Fc fusion vector to carry out in vitro recombination using Gibson Assembly Mastermix (New England Biolabs, USA), which was subsequently used for heat shock transformation into *E. coli* strain NEB® 5-alpha. This shuffling with wildtype sequence yielded SEQ ID NOS: 4-10.

**[0623]** A second library of random mutations was generated via error prone PCR using the clones from Gen1 selection as template. This library, described as a Gen2, was constructed using the same process previously described except that template DNA was composed of a pool of Gen1 clones instead of wildtype CTLA-4 ECD DNA. The yeast library was screened via iterative rounds of FACS sorting, alternating between rICOSL-Fc and rCD86-Fc, to generate multiple pools of clones. As before, yeast pools were analyzed for binding via FACS. Based on the binding to rICOSL-Fc, rCD86-Fc, rCD80-Fc by FACS, several pools were selected for PCR cloning into the Fc vector. Subsequent sequence analysis and protein production were performed as described for Gen1.

**[0624]** Selection outputs were reformatted as immunomodulatory proteins containing an affinity-modified (variant) ECD of CTLA-4 fused to an Fc molecule (variant ECD-Fc fusion molecules). To generate recombinant immunomodulatory proteins that are Fc fusion proteins containing an ECD of CTLA-4 with at least one affinity-modified domain (e.g., variant CTLA-4 ECD-Fc), the encoding DNA was generated to encode a protein as follows: variant (mutant) ECD followed by a linker of 7 amino acids (GSGGGGS; SEQ ID NO:590) followed by a human IgG1 Fc containing the mutations L234A, L235E and G237A by EU numbering. Since the construct does not include any antibody light chains that can form a covalent bond with a cysteine, the human IgG1 Fc also contained replacement of the cysteine residues to a serine residue at position 220 (C220S) by EU numbering (corresponding to position 5 (C5S) with reference to the wild-type or unmodified Fc set forth in SEQ ID NO: 586). The Fc region also lacked the C-terminal lysine at position 447 (designated K447del) normally encoded in the wild type human IgG1 constant region gene (corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 586). The effectorless (inert) IgG1 Fc in the fusion constructs is set forth in SEQ ID O:589:

SEQ ID NO: 589
EPKSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

**[0625]** Output cells from final flow cytometric CTLA-4 sorts were grown to terminal density in SCD-Leu medium. Plasmid DNA from each output was isolated using a yeast plasmid DNA isolation kit

(Zymoresearch, USA). For Fc fusions, PCR primers with added restriction sites suitable for cloning into the Fc fusion vector of choice were used to batch-amplify from the plasmid DNA preps the coding DNA for the mutant target ECDs. After restriction digestion, the PCR products were ligated into an appropriate Fc fusion vector followed by heat shock transformation into strain XL1-Blue *E. coli* (Agilent, USA) or NEB® 5-alpha (New England Biolabs) as directed by supplier. Alternatively, the outputs were PCR amplified with primers containing 40 bp overlap regions on either end with a modified Fc fusion vector to carry out in vitro recombination using Gibson Assembly Mastermix (New England Biolabs, USA), which was subsequently used for heat shock transformation into *E. coli* strain NEB® 5-alpha. An exemplary Fc fusion vector is pFUSE-hIgG1-Fc2 (InvivoGen, USA).

**[0626]** Dilutions of transformation reactions were plated on LB-agar containing 100 μg/mL carbenicillin (Teknova, USA) to isolate single colonies for selection. Up to 96 colonies from each transformation were then grown in 96-well plates to saturation overnight at 37 °C in LB-broth (cat. # L8112, Teknova, USA), and a small aliquot from each well was submitted for DNA sequencing of the ECD insert in order to identify the mutation(s) in all clones. Sample preparation for DNA sequencing was carried out using protocols provided by the service provider (Genewiz; South Plainfield, NJ). After removal of the sample for DNA sequencing, glycerol was added to the remaining cultures for a final glycerol content of 25%, and plates were stored at -20 °C for future use as master plates (see below). Alternatively, samples for DNA sequencing were generated by replica plating from grown liquid cultures to solid agar plates using a disposable 96-well replicator (VWR, USA). These plates were incubated overnight to generate growth patches and the plates were submitted to Genewiz for DNA sequencing following their specifications.

**[0627]** After analysis of Genewiz-generated DNA sequencing data, clones of interest were recovered from master plates and individually grown to saturation in 5 mL liquid LB-broth containing 100 μg/mL carbenicillin (Teknova, USA) and 2 mL of each culture were then used for preparation of approximately 10 μg of miniprep plasmid DNA of each clone using a standard kit such as the PureYield Plasmid Miniprep System (Promega, USA). Identification of clones of interest generally involved the following steps. First, DNA sequence data files were downloaded from the Genewiz website. All sequences were then manually curated so that they start at the beginning of the ECD coding region. The Genewiz sequences were processed to generate alignments using Ugene software (http://ugene.net).

**[0628]** Clones of interest were then identified using the following criteria: 1) identical clone occurs at least two times in the alignment, and 2) a mutation occurs at least two times in the alignment and preferably in distinct clones. Clones that met at least one of these criteria were enriched by the sorting process mostly likely due to improved binding.

**[0629]** The Fc-fusion proteins, containing variant ECDs of CTLA-4, were generated by high throughput expression and purification. Recombinant variant Fc fusion proteins were produced from

suspension-adapted human embryonic kidney (HEK) 293 cells using the Expi293 expression system (Invitrogen, USA). 4µg of each plasmid DNA were added to 200µL Opti-MEM (Invitrogen, USA) at the same time as 10.8 µL ExpiFectamine were separately added to another 200µL Opti-MEM. After 5 minutes, the 200µL of plasmid DNA was mixed with the 200µL of ExpiFectamine and was further incubated for an additional 20 minutes before adding this mixture to cells. Ten million Expi293 cells were dispensed into separate wells of a sterile 10 mL, conical bottom, deep 24-well growth plate (Thomson Instrument Company, USA) in a volume of 4 mL Expi293 media (Invitrogen, USA). Plates were shaken for 5 days at 120 RPM in a mammalian cell culture incubator set to 95% humidity and 8% $CO_2$. Following a 5-day incubation, cells were pelleted and culture supernatants were removed.

**[0630]** Protein was purified from supernatants using a high throughput 96-well Protein A purification kit using the manufacturer's protocol (Catalog number 45202, Life Technologies, USA). Resulting elution fractions were buffer-exchanged into PBS using Zeba 96-well spin desalting plate (Catalog number 89807, Life Technologies, USA) using the manufacturer's protocol. Purified protein was quantitated using 280 nm absorbance measured by Nanodrop instrument (Thermo Fisher Scientific, USA), and protein purity was assessed by loading 5 µg of protein on NUPAGE pre-cast, polyacrylamide gels (Life Technologies, USA) under denaturing and reducing conditions and subsequent gel electrophoresis. Proteins were visualized in gel using standard Coomassie staining.

**[0631]** Amino acid substitutions in selected CTLA-4 vIgDs that were identified by the selection are set forth in Table 3. Selected CTLA-4 vIgDs, formatted as Fc fusion proteins, were tested for binding and functional activity as described below.

### A. *Functional Characterization*

**[0632]** Binding studies were performed to assess specificity and affinity of CTLA-4 vIgD-Fc fusion proteins for binding partners CD80, CD86, and ICOSL. The Fc-fusion variant proteins were further characterized for bioactivity in human primary T cells *in vitro* assays.

#### *1. Binding*

**[0633]** To produce cells expressing a binding partner, full-length mammalian surface expression constructs for each of human CD80, CD86, and ICOSL were designed in pcDNA3.1 expression vectors (Life Technologies) and sourced from Genscript, USA. Binding studies were carried out using the Expi293F transient transfection system (Life Technologies, USA) described above. The number of cells needed for the experiment was determined, and the appropriate 30 mL scale of transfection was performed using the manufacturer's suggested protocol. For each counter structure or mock 30 mL transfection, 75 million Expi293F cells were incubated with 30 µg expression construct DNA and 1.5 mL diluted ExpiFectamine 293 reagent for 48 hours, at which point cells were harvested for staining.

**[0634]** For flow cytometric analysis, 200,000 cells of appropriate transient transfection or negative control were plated in 96 well round bottom plates. Cells were spun down and suspended in staining buffer (PBS (phosphate buffered saline), 1% BSA (bovine serum albumin), and 0.1% sodium azide) for 20 minutes to block non-specific binding. Afterwards, cells were centrifuged again and suspended in staining buffer containing 100 nM to 100 pM CTLA-4 IgSF variant Fc fusion protein in 50 µL. Primary staining was performed for 45 minutes, before washing cells in staining buffer twice. For CD86 transfections, bound protein was detected with PE-conjugated anti-human Fc (Jackson ImmunoResearch, USA) diluted 1:150 in 50 µL staining buffer incubated for 30 minutes. For CD80 and ICOSL transfections, bound protein was captured with anti-CTLA-4 antibody (Biolegend, USA) diluted 1:130 in 50 µL staining buffer. After a 30 minute incubation, cells were washed twice and detected with PE-conjugated anti-mouse Fc (Jackson ImmunoResearch, USA) diluted 1:150 in 50 µL for an additional 30 minute incubation. Cells were washed twice to remove unbound conjugated antibodies, fixed in 2% formaldehyde/PBS, and analyzed on a FACScan (Becton Dickinson, USA) or a Hypercyt flow cytometer (Intellicyte, USA).

**[0635]** Mean Fluorescence Intensity (MFI) was calculated for each transfectant and negative parental line with Cell Quest Pro software (Becton Dickinson, USA) or Forcyte software (Intellicyt, USA).

### *2. Cytokine Production in Anti-CD3 Costimulation Assays*

**[0636]** Soluble CTLA-4-Fc bioactivity was tested in a human Mixed Lymphocyte Reaction (MLR). Human primary dendritic cells (DC) generated by culturing monocytes isolated from PBMC (BenTech Bio, USA) in vitro for 7 days with 50 ng/mL rIL-4 (R&D Systems, USA) and 80ng/mL rGM-CSF (R&D Systems, USA) in Ex-Vivo 15 media (Lonza, Switzerland). On days 3 and 5, half of the media was removed and replaced with fresh media containing 50 ng/mL rIL-4 and 80 ng/mL rGM-CSF. To fully induce DC maturation, lipopolysaccharide (LPS) (InvivoGen Corp., USA) was added at 100 ng/mL to the DC cultures on day 6 and cells were incubated for an additional 24 hours. Approximately, 10,000 matured DC and 100,000 purified allogeneic CD3+ T cells (BenTech Bio, USA) were co-cultured with CTLA-4 variant Fc fusion proteins and controls in 96 well round bottom plates in 200µl final volume of Ex-Vivo 15 media. On day 4 -5, IFN-gamma secretion in culture supernatants was analyzed using the Human IFN-gamma Duoset ELISA kit (R&D Systems, USA). Optical density was measured on a BioTek Cytation Multimode Microplate Reader (BioTek Corp., USA) and quantitated against titrated rIFN-gamma standard included in the IFN-gamma Duo-set kit (R&D Systems, USA).

### *3. Results*

**[0637]** The results of the binding and costimulatory bioactivity assays described above for the variant and unmodified CTLA-4 polypeptides are summarized in Tables E1-E3. The values for binding

CD80, CD86, and ICOSL (MFI) and interferon-gamma secretion [pg/mL] are provided in addition to the relative ratio, as compared to the corresponding binding and secretion of the unmodified CTLA-4 polypeptide (ΔWT) for each experiment. Relative ratios for binding that were substantially below 0.1, are reported as 0. In addition, MLR data for variants in which the variant suppressed the secretion of interferon gamma to undetectable levels also is reported as 0.

**Table E1. Binding and costimulatory bioactivity of variant CTLA-4-Fc polypeptides**

| Mutations | SEQ ID NO (ECD) | Binding | | | MLR |
|---|---|---|---|---|---|
| | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | IFN-γ [pg/mL] ( Δ WT ) |
| L12P/A26T/L63P/L98Q/Y105L | 3 | 829 ( 0.2 ) | 761890 ( 1.1 ) | 873 ( 0.5 ) | 216 ( 0.3 ) |
| L12P/A26T | 5 | 1024 ( 0.2 ) | 276276 ( 0.4 ) | 928 ( 0.6 ) | 850 ( 1.3 ) |
| L12P/A26T/L63P | 6 | 2400 ( 0.5 ) | 500345 ( 0.7 ) | 891 ( 0.5 ) | 671 ( 1.0 ) |
| L63P/L98Q/Y105L | 7 | 4718 ( 1.0 ) | 410571 ( 0.6 ) | 1802 ( 1.1 ) | 124 ( 0.2 ) |
| L98Q/Y105L | 8 | 3863 ( 0.8 ) | 685365 ( 1.0 ) | 1186 ( 0.7 ) | 124 ( 0.2 ) |
| L63P | 9 | 3932 ( 0.8 ) | 595807 ( 0.8 ) | 966 ( 0.6 ) | 261 ( 0.4 ) |
| L98R/N110K | 10 | 2110 ( 0.4 ) | 665012 ( 0.9 ) | 1046 ( 0.6 ) | 344 ( 0.5 ) |
| WT CTLA-4 | 1 | 4775 ( 1.0 ) | 708753 ( 1.0 ) | 1664 ( 1.0 ) | 662 ( 1.0 ) |

**Table E2. Binding and costimulatory bioactivity of variant CTLA-4-Fc polypeptides**

| Mutations | SEQ ID NO (ECD) | Binding | | | MLR |
|---|---|---|---|---|---|
| | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | IFN-γ [pg/mL] ( Δ WT ) |
| L12P/A26T/L63P/L98Q/M99L/Y105L | 11 | 2026 ( 0.4 ) | 33068 ( 0.9 ) | 1222 ( 0.7 ) | 569 ( 1.5 ) |
| E33M/Q82H/L98Q/M99L/Y105L | 12 | 1098 ( 0.2 ) | 35506 ( 1.0 ) | 1792 ( 1.1 ) | 253 ( 0.7 ) |
| L63P/S72G/L98Q/M99L/Y105L | 13 | 2591 ( 0.5 ) | 33477 ( 0.9 ) | 1604 ( 1.0 ) | 586 ( 1.6 ) |
| S14N/R16C/I18T/M56K/T61A/L63P/A86T/M99L | 14 | 3773 ( 0.8 ) | 30572 ( 0.8 ) | 990 ( 0.6 ) | 441 ( 1.2 ) |
| S27P/M56K/L63P/S72G/S73R/T89A/M99L/Y105L/I117M | 15 | 1982 ( 0.4 ) | 33467 ( 0.9 ) | 1354 ( 0.8 ) | 426 ( 1.1 ) |
| M56K/L63P/N75D/V96I/M99L/Y105L/L106I | 16 | 3775 ( 0.8 ) | 31296 ( 0.9 ) | 1719 ( 1.0 ) | 583 ( 1.6 ) |

| Table E2. Binding and costimulatory bioactivity of variant CTLA-4-Fc polypeptides | | | | | |
|---|---|---|---|---|---|
| | SEQ ID NO (ECD) | Binding | | | MLR |
| Mutations | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | IFN-γ [pg/mL] ( Δ WT ) |
| L63P/S72G/Y105L | 17 | 3831 ( 0.8 ) | 32160 ( 0.9 ) | 1362 ( 0.8 ) | 123 ( 0.3 ) |
| L63P/L98Q/M99L/Y105L/ I117M | 18 | 2635 ( 0.6 ) | 32564 ( 0.9 ) | 1761 ( 1.1 ) | 539 ( 1.4 ) |
| L63P/S72G/L98Q/M99L/Y 105L/L106I/I117L | 19 | 2463 ( 0.5 ) | 32830 ( 0.9 ) | 1930 ( 1.2 ) | 603 ( 1.6 ) |
| A26T/L63P/S72G/L98Q/Y 105L/L106I/I117L | 20 | 3576 ( 0.7 ) | 31549 ( 0.9 ) | 939 ( 0.6 ) | 83 ( 0.2 ) |
| L63P/L98Q/V116A | 21 | 2772 ( 0.6 ) | 32657 ( 0.9 ) | 1033 ( 0.6 ) | 298 ( 0.8 ) |
| G29W/L98Q/M99L/Y105L | 22 | 1772 ( 0.4 ) | 32977 ( 0.9 ) | 6183 ( 3.7 ) | 745 ( 2.0 ) |
| T37S/M56V/L98Q/Y105L | 23 | 2115 ( 0.4 ) | 27628 ( 0.8 ) | 881 ( 0.5 ) | 148 ( 0.4 ) |
| A26T/Y54F/M56K/M99L/ Y105L | 24 | 1526 ( 0.3 ) | 28149 ( 0.8 ) | 1113 ( 0.7 ) | 552 ( 1.5 ) |
| L12P/I18T/A26T/M55T/T6 9S/S72G/M99L/Y105L | 25 | 1577 ( 0.3 ) | 25936 ( 0.7 ) | 931 ( 0.6 ) | 944 ( 2.5 ) |
| V22I/L63P/L98Q/Y105L/I 117M | 26 | 2802 ( 0.6 ) | 27629 ( 0.8 ) | 1013 ( 0.6 ) | 103 ( 0.3 ) |
| A26T/L63P/S72G/L98Q/M 99L/Y105L | 27 | 2899 ( 0.6 ) | 26407 ( 0.7 ) | 1759 ( 1.1 ) | 195 ( 0.5 ) |
| I18T/T61R/L63P/S72G/L9 8Q/M99L/P102L/Y105L | 190 | 1140 ( 0.2 ) | 46974 ( 1.3 ) | 935 ( 0.6 ) | 714 ( 1.9 ) |
| E33M/A42T/L98Q/Y105L | 28 | 1623 ( 0.3 ) | 27354 ( 0.7 ) | 1675 ( 1.0 ) | 638 ( 1.7 ) |
| M55T/E97Q/M99L/Y105F | 29 | 906 ( 0.2 ) | 6249 ( 0.2 ) | 1037 ( 0.6 ) | 575 ( 1.5 ) |
| M55T/S72G/L98Q/M99L/ Y105L | 30 | 1940 ( 0.4 ) | 30594 ( 0.8 ) | 2313 ( 1.4 ) | 594 ( 1.6 ) |
| R16C/G29W/E33V/M55T/ L63P/L98Q/Y105L | 31 | 2678 ( 0.6 ) | 28858 ( 0.8 ) | 1480 ( 0.9 ) | 144 ( 0.4 ) |
| L12P/A26T/L63P/L98Q/Y 105L/L106I | 32 | 2318 ( 0.5 ) | 28463 ( 0.8 ) | 879 ( 0.5 ) | 127 ( 0.3 ) |
| M56L/L63P/L98Q/Y105L/ L106I/I117L | 33 | 3487 ( 0.7 ) | 32054 ( 0.9 ) | 963 ( 0.6 ) | 72 ( 0.2 ) |
| S15P/I18V/M56T/L98Q/M 99L/Y105L | 34 | 1445 ( 0.3 ) | 33793 ( 0.9 ) | 1505 ( 0.9 ) | 622 ( 1.7 ) |
| I18T/G29W/L63P/L98Q/Y 105L | 35 | 10109 ( 2.1 ) | 29367 ( 0.8 ) | 1711 ( 1.0 ) | 50 ( 0.1 ) |
| L63P/Q82H/L98Q/M99L/ Y105L | 36 | 2777 ( 0.6 ) | 31740 ( 0.9 ) | 2110 ( 1.3 ) | 723 ( 1.9 ) |
| L98Q/M99L/Y105L/L106I/ I117T | 37 | 1117 ( 0.2 ) | 28174 ( 0.8 ) | 1081 ( 0.6 ) | 944 ( 2.5 ) |
| L98Q/M99L/Y105L/L106I/ Y115N | 38 | 1074 ( 0.2 ) | 27514 ( 0.7 ) | 939 ( 0.6 ) | 322 ( 0.9 ) |

| Table E2. Binding and costimulatory bioactivity of variant CTLA-4-Fc polypeptides | | | | | |
|---|---|---|---|---|---|
| | SEQ ID NO (ECD) | Binding | | | MLR |
| Mutations | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | IFN-γ [pg/mL] ( Δ WT ) |
| M55T/L63P/T71I/M99L/Y105L | 39 | 2900 ( 0.6 ) | 24010 ( 0.7 ) | 1125 ( 0.7 ) | 384 ( 1.0 ) |
| A26T/T53S/M56K/L63P/L98Q/Y105L | 40 | 3352 ( 0.7 ) | 23688 ( 0.6 ) | 1042 ( 0.6 ) | 88 ( 0.2 ) |
| I18T/A26T/L63P/Q82R/L98Q/Y105L | 41 | 3650 ( 0.8 ) | 26133 ( 0.7 ) | 923 ( 0.6 ) | 105 ( 0.3 ) |
| L12H/M55T/E59D/L63P/M99L | 42 | 2877 ( 0.6 ) | 26206 ( 0.7 ) | 876 ( 0.5 ) | 619 ( 1.7 ) |
| I18T/L63P/S72G/L98Q/Y105L/I108V | 43 | 2706 ( 0.6 ) | 26196 ( 0.7 ) | 960 ( 0.6 ) | 62 ( 0.2 ) |
| I18T/L63P/S72G/L98Q/M99L/Y105L | 44 | 2442 ( 0.5 ) | 29111 ( 0.8 ) | 2489 ( 1.5 ) | 817 ( 2.2 ) |
| T61A/L63P/S72G/L98Q/M99L/Y105L | 45 | 2505 ( 0.5 ) | 32390 ( 0.9 ) | 1987 ( 1.2 ) | 944 ( 2.5 ) |
| V38I/L63P/S72G/L98Q/M99L/Y105L | 46 | 3433 ( 0.7 ) | 33373 ( 0.9 ) | 2410 ( 1.4 ) | 846 ( 2.3 ) |
| L63P/S72G/I93L/L98Q/M99L/Y105L | 47 | 3282 ( 0.7 ) | 32885 ( 0.9 ) | 2277 ( 1.4 ) | 897 ( 2.4 ) |
| L12I/M55T/M56V/I67T/M99L/L106R/I108F | 48 | 2917 ( 0.6 ) | 31744 ( 0.9 ) | 2485 ( 1.5 ) | 842 ( 2.3 ) |
| I18N/A26T/L63H/T89A/L98Q/M99L/Y105L | 49 | 1943 ( 0.4 ) | 31558 ( 0.9 ) | 2175 ( 1.3 ) | 689 ( 1.8 ) |
| I18T/E48R/L63P/T69S/L98Q/Y105L/N110Y | 50 | 1086 ( 0.2 ) | 23508 ( 0.6 ) | 1124 ( 0.7 ) | 645 ( 1.7 ) |
| I18N/L63P/S72T/M87T/L98Q/Y105L/N110S | 51 | 1998 ( 0.4 ) | 36385 ( 1.0 ) | 1032 ( 0.6 ) | 73 ( 0.2 ) |
| G29W/M56T/L63P/L98Q/Y105L/L106I/I117L | 52 | 3308 ( 0.7 ) | 32787 ( 0.9 ) | 1258 ( 0.8 ) | 78 ( 0.2 ) |
| G29W/N58S/L63P/M87T/L98Q/M99L/Y105L | 53 | 3381 ( 0.7 ) | 32622 ( 0.9 ) | 3622 ( 2.2 ) | 578 ( 1.6 ) |
| G29W/N58S/L63P/D64N/L98Q/M99L/Y105L | 54 | 3750 ( 0.8 ) | 33612 ( 0.9 ) | 2158 ( 1.3 ) | 227 ( 0.6 ) |
| I18T/L63P/S72G/M87K/L98Q/M99L/Y105L | 55 | 2925 ( 0.6 ) | 35032 ( 1.0 ) | 1999 ( 1.2 ) | 679 ( 1.8 ) |
| WT CTLA-4 | 1 | 4775 ( 1.0 ) | 36785 ( 1.0 ) | 1664 ( 1.0 ) | 373 ( 1.0 ) |

| Table E3. Binding and costimulatory bioactivity of variant CTLA-4-Fc polypeptides | | | | | |
|---|---|---|---|---|---|
| | SEQ ID NO (ECD) | Binding | | | MLR |
| Mutations | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | IFN-γ [pg/mL] ( Δ WT ) |
| M56V | 56 | 2688 ( 0.6 ) | 36766 ( 0.1 ) | 822 ( 0.5 ) | 176 ( 1.3 ) |

| Table E3. Binding and costimulatory bioactivity of variant CTLA-4-Fc polypeptides | | | | | |
|---|---|---|---|---|---|
| | SEQ ID NO (ECD) | Binding | | | MLR |
| Mutations | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | IFN-γ [pg/mL] ( Δ WT ) |
| L63P/K95R | 57 | 2914 ( 0.6 ) | 33412 ( 0.0 ) | 819 ( 0.5 ) | 165 ( 1.2 ) |
| L63P/L98Q | 58 | 2830 ( 0.6 ) | 31416 ( 0.0 ) | 885 ( 0.5 ) | 229 ( 1.6 ) |
| L98Q/M99L/Y105L | 59 | 1472 ( 0.3 ) | 33977 ( 0.0 ) | 1541 ( 0.9 ) | 325 ( 2.3 ) |
| L63P/M87K/M99L/L106R | 60 | 3329 ( 0.7 ) | 61526 ( 0.1 ) | 2540 ( 1.5 ) | 531 ( 3.8 ) |
| L63P/M99L/Y105L/I108F | 61 | 2142 ( 0.4 ) | 32781 ( 0.0 ) | 3759 ( 2.3 ) | 1053 ( 7.5 ) |
| V10A/L63P/L98Q/Y105L | 62 | 3148 ( 0.7 ) | 34595 ( 0.0 ) | 869 ( 0.5 ) | 141 ( 1.0 ) |
| M56T/L91R/L98Q/Y105L | 63 | 1713 ( 0.4 ) | 33645 ( 0.0 ) | 1128 ( 0.7 ) | 0 ( 0.0 ) |
| A26T/L63P/M87V/N110K/I117E | 64 | 2909 ( 0.6 ) | 31487 ( 0.0 ) | 973 ( 0.6 ) | 426 ( 3.0 ) |
| G29W/L63P/L98Q/M99L/Y105L | 65 | 5165 ( 1.1 ) | 37721 ( 0.1 ) | 3023 ( 1.8 ) | 438 ( 3.1 ) |
| A26T/V46E/L63P/D65G/L98Q | 66 | 5009 ( 1.0 ) | 38407 ( 0.1 ) | 888 ( 0.5 ) | 273 ( 1.9 ) |
| G29W/N58S/L63P/L98Q/Y105L | 67 | 15619 ( 3.3 ) | 34897 ( 0.0 ) | 1374 ( 0.8 ) | 0 ( 0.0 ) |
| G29W/E59G/L63P/L98Q/Y105L | 68 | 3214 ( 0.7 ) | 32786 ( 0.0 ) | 1148 ( 0.7 ) | 0 ( 0.0 ) |
| L12H/L63P/S72G/L98Q/Y105L | 69 | 2034 ( 0.4 ) | 31843 ( 0.0 ) | 857 ( 0.5 ) | 87 ( 0.6 ) |
| A6T/A26T/M55T/M99L/Y105L | 70 | 1429 ( 0.3 ) | 33589 ( 0.0 ) | 938 ( 0.6 ) | 472 ( 3.4 ) |
| A26T/L63P/D65G/L98Q/M99L/Y105L | 71 | 2324 ( 0.5 ) | 33672 ( 0.0 ) | 2200 ( 1.3 ) | 264 ( 1.9 ) |
| V10A/L63P/D64V/S72G/L98Q/M99L/Y105L | 72 | 2598 ( 0.5 ) | 33868 ( 0.0 ) | 2502 ( 1.5 ) | 904 ( 6.4 ) |
| L12P/G29W/D43N/N58S/L63P/L98Q/M99L/Y105L | 73 | 1486 ( 0.3 ) | 30004 ( 0.0 ) | 1276 ( 0.8 ) | 352 ( 2.5 ) |
| I18V/A26T/L63P/D64E/L98Q/Y105L/L106R/N110K | 74 | 4096 ( 0.9 ) | 30852 ( 0.0 ) | 17220 ( 10.3 ) | 0 ( 0.0 ) |
| A19V/G29W/R35K/L63P/L98Q/M99L/Y105L | 75 | 2349 ( 0.5 ) | 33255 ( 0.0 ) | 3119 ( 1.9 ) | 445 ( 3.2 ) |
| L12P/A26T/L63P/S72G/T89M/L98Q/M99L/Y105L | 76 | 1833 ( 0.4 ) | 924222 ( 1.3 ) | 919 ( 0.6 ) | 536 ( 3.8 ) |
| P28L/E33V/L63P/S72G/L98R/M99L/Y105L | 77 | 1441 ( 0.3 ) | 782025 ( 1.1 ) | 966 ( 0.6 ) | 535 ( 3.8 ) |

| Table E3. Binding and costimulatory bioactivity of variant CTLA-4-Fc polypeptides | | | | | |
|---|---|---|---|---|---|
| | SEQ ID NO (ECD) | Binding | | | MLR |
| Mutations | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | IFN-γ [pg/mL] ( Δ WT ) |
| E24Q/L63P/S72G/L98Q/M99L/Y105L | 78 | 2864 ( 0.6 ) | 729343 ( 1.0 ) | 1080 ( 0.6 ) | 867 ( 6.2 ) |
| I18T/G29R/L63P/S72G/L98Q/M99L/Y105L | 79 | 3592 ( 0.8 ) | 857127 ( 1.2 ) | 1014 ( 0.6 ) | 366 ( 2.6 ) |
| L63P/L98Q/M99L/Y105L | 80 | 2662 ( 0.6 ) | 618249 ( 0.9 ) | 868 ( 0.5 ) | 944 ( 6.7 ) |
| Q41L/Y54F/M56K/M99L/I108F | 81 | 2570 ( 0.5 ) | 703731 ( 1.0 ) | 940 ( 0.6 ) | 408 ( 2.9 ) |
| S72G/L98Q/M99L/Y105L/I117T | 82 | 1374 ( 0.3 ) | 863538 ( 1.2 ) | 968 ( 0.6 ) | 221 ( 1.6 ) |
| M56R/L63P/L98Q/M99L/Y105L | 83 | 2546 ( 0.5 ) | 911035 ( 1.3 ) | 839 ( 0.5 ) | 1198 ( 8.5 ) |
| E33M/L63P/S72G/L98Q/Y105L | 84 | 1532 ( 0.3 ) | 518203 ( 0.7 ) | 999 ( 0.6 ) | 1220 ( 8.7 ) |
| L63P/L98Q/M99L/Y105L/L106I | 85 | 2814 ( 0.6 ) | 1007606 ( 1.4 ) | 1004 ( 0.6 ) | 773 ( 5.5 ) |
| A26T/M55R/L98Q/M99L/Y105L | 86 | 2324 ( 0.5 ) | 520232 ( 0.7 ) | 986 ( 0.6 ) | 468 ( 3.3 ) |
| L63P/S72G/M87A/L98Q/Y105L | 87 | 2769 ( 0.6 ) | 349875 ( 0.5 ) | 875 ( 0.5 ) | 202 ( 1.4 ) |
| A26D/S72G/L98Q/M99L/Y105L | 88 | 5409 ( 1.1 ) | 578704 ( 0.8 ) | 1235 ( 0.7 ) | 1097 ( 7.8 ) |
| V22A/L63P/L98Q/M99L/Y105L/P119H | 89 | 2820 ( 0.6 ) | 642849 ( 0.9 ) | 992 ( 0.6 ) | 1174 ( 8.4 ) |
| A26T/M55T/L63P/L98Q/M99L/Y105L | 90 | 3203 ( 0.7 ) | 850654 ( 1.2 ) | 875 ( 0.5 ) | 1096 ( 7.8 ) |
| E33V/A42S/M55T/L98Q/M99L/Y105L | 91 | 2195 ( 0.5 ) | 929792 ( 1.3 ) | 1043 ( 0.6 ) | 1478 ( 10.5 ) |
| G29W/N58S/L63P/Q82R/L98Q/Y105L | 92 | 18277 ( 3.8 ) | 950639 ( 1.3 ) | 1463 ( 0.9 ) | 0 ( 0.0 ) |
| E33M/L63P/S72G/L98Q/Y105L/I117L | 93 | 2293 ( 0.5 ) | 912480 ( 1.3 ) | 907 ( 0.5 ) | 586 ( 4.2 ) |
| A26T/I67N/S72G/L98Q/M99L/Y105L | 94 | 1740 ( 0.4 ) | 976150 ( 1.4 ) | 948 ( 0.6 ) | 1331 ( 9.5 ) |
| L12F/A26T/L63P/L98Q/Y105L/L106R | 95 | 2186 ( 0.5 ) | 984573 ( 1.4 ) | 867 ( 0.5 ) | 1286 ( 9.2 ) |
| S20N/A26T/L63P/L98Q/M99L/Y105L | 96 | 3707 ( 0.8 ) | 941466 ( 1.3 ) | 1020 ( 0.6 ) | 1879 ( 13.4 ) |
| G29W/T61I/L63P/S72G/L98Q/M99L/Y105L | 97 | 3446 ( 0.7 ) | 842791 ( 1.2 ) | 1024 ( 0.6 ) | 718 ( 5.1 ) |
| G29W/N58S/L63P/T69I/L98Q/M99L/Y105L | 98 | 4558 ( 1.0 ) | 841939 ( 1.2 ) | 1945 ( 1.2 ) | 1036 ( 7.4 ) |
| L12P/L63P/S72G/L98Q/M99L/Y105L/L106N | 99 | 2991 ( 0.6 ) | 854863 ( 1.2 ) | 894 ( 0.5 ) | 0 ( 0.0 ) |

| Table E3. Binding and costimulatory bioactivity of variant CTLA-4-Fc polypeptides | | | | | |
|---|---|---|---|---|---|
| | SEQ ID NO (ECD) | Binding | | | MLR |
| Mutations | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | IFN-γ [pg/mL] ( Δ WT ) |
| L63P/T69A/L98Q/M99L/Y105L/L106R/V116A | 100 | 3984 ( 0.8 ) | 831276 ( 1.2 ) | 1765 ( 1.1 ) | 0 ( 0.0 ) |
| G29W/N58S/L63P/S72G/L98Q/Y105L | 101 | 4262 ( 0.9 ) | 860194 ( 1.2 ) | 1445 ( 0.9 ) | 0 ( 0.0 ) |
| G29W/L63P/D65G/S72G/L98Q/Y105L | 102 | 3399 ( 0.7 ) | 854339 ( 1.2 ) | 954 ( 0.6 ) | 0 ( 0.0 ) |
| T53S/M56V/L98Q/Y105L | 103 | 3860 ( 0.8 ) | 875378 ( 1.2 ) | 1376 ( 0.8 ) | 0 ( 0.0 ) |
| L63P/S72G/L98Q/Y105L | 104 | 3451 ( 0.7 ) | 892268 ( 1.3 ) | 1486 ( 0.9 ) | 0 ( 0.0 ) |
| I18A/L63P/S72G/L98Q/Y105L | 105 | 3542 ( 0.7 ) | 637802 ( 0.9 ) | 1240 ( 0.7 ) | 0 ( 0.0 ) |
| G29W/T53S/M56K/L63P/L98Q/Y105L | 106 | 3347 ( 0.7 ) | 794165 ( 1.1 ) | 1914 ( 1.2 ) | 179 ( 1.3 ) |
| I18V/G29W/L63P/S72G/L98Q/Y105L | 107 | 4064 ( 0.9 ) | 797318 ( 1.1 ) | 1351 ( 0.8 ) | 0 ( 0.0 ) |
| G29W/L63P/S72G/L98Q/Y105L/L106I | 108 | 4303 ( 0.9 ) | 829524 ( 1.2 ) | 1474 ( 0.9 ) | 0 ( 0.0 ) |
| G29W/L63P/I67V/S72G/L98Q/Y105L | 109 | 3993 ( 0.8 ) | 769557 ( 1.1 ) | 1053 ( 0.6 ) | 0 ( 0.0 ) |
| G29W/M55V/E59G/L63P/L98Q/Y105L | 110 | 4174 ( 0.9 ) | 427427 ( 0.6 ) | 1248 ( 0.7 ) | 0 ( 0.0 ) |
| G29W/L63P/S72G/L98Q/Y105L/I117L | 111 | 3794 ( 0.8 ) | 502885 ( 0.7 ) | 1853 ( 1.1 ) | 0 ( 0.0 ) |
| L63P/S72G/L98Q/Y105L/L106I/I117L | 112 | 3811 ( 0.8 ) | 789352 ( 1.1 ) | 1885 ( 1.1 ) | 37 ( 0.3 ) |
| L12F/R16H/G29W/M56T/L98Q/Y105L | 113 | 6575 ( 1.4 ) | 919746 ( 1.3 ) | 2615 ( 1.6 ) | 0 ( 0.0 ) |
| L12P/G29W/L63P/S72G/L98Q/Y105L | 114 | 4012 ( 0.8 ) | 783049 ( 1.1 ) | 1001 ( 0.6 ) | 155 ( 1.1 ) |
| L12P/G29W/L63P/S72G/L98Q/Y105L/L106I | 115 | 4347 ( 0.9 ) | 662327 ( 0.9 ) | 1219 ( 0.7 ) | 195 ( 1.4 ) |
| G29W/L63P/S72G/L98Q/Y105L/L106I/I117L | 116 | 3242 ( 0.7 ) | 702231 ( 1.0 ) | 1205 ( 0.7 ) | 133 ( 0.9 ) |
| A26T/T53S/L63P/L98Q/Y105L/L106I/I117L | 118 | 4853 ( 1.0 ) | 713974 ( 1.0 ) | 2111 ( 1.3 ) | 0 ( 0.0 ) |
| G29W/N58S/L63P/S72G/M87V/L98Q/Y105L | 119 | 4044 ( 0.8 ) | 818528 ( 1.2 ) | 1572 ( 0.9 ) | 0 ( 0.0 ) |
| G29W/S72G/Q76R/L98Q/Y105L/L106I/Q113H | 120 | 2421 ( 0.5 ) | 842313 ( 1.2 ) | 2147 ( 1.3 ) | 1129 ( 8.1 ) |
| G29W/N58S/L63P/S72G/L98Q/Y105L/L106V | 121 | 1233 ( 0.3 ) | 931184 ( 1.3 ) | 1045 ( 0.6 ) | 844 ( 6.0 ) |
| A26T/L63P/L98Q/M99L/Y105L | 122 | 3095 ( 0.6 ) | 762915 ( 1.1 ) | 1863 ( 1.1 ) | 1059 ( 7.6 ) |

| Table E3. Binding and costimulatory bioactivity of variant CTLA-4-Fc polypeptides | | | | | |
|---|---|---|---|---|---|
| | SEQ ID NO (ECD) | Binding | | | MLR |
| Mutations | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | IFN-γ [pg/mL] ( Δ WT ) |
| G29W/N58D/I67V/L98Q/M99L/Y105L | 123 | 2460 ( 0.5 ) | 898877 ( 1.3 ) | 4222 ( 2.5 ) | 373 ( 2.7 ) |
| I67V/S72G/Q82H/T89A/L98Q/M99L/Y105L | 124 | 1729 ( 0.4 ) | 865295 ( 1.2 ) | 5692 ( 3.4 ) | 786 ( 5.6 ) |
| S72G/R85G/L98Q/M99L/Y105L/L106I | 125 | 1439 ( 0.3 ) | 905813 ( 1.3 ) | 4653 ( 2.8 ) | 915 ( 6.5 ) |
| L63P/L98Q/M99L/Y105L | 80 | 2787 ( 0.6 ) | 824331 ( 1.2 ) | 1723 ( 1.0 ) | 692 ( 4.9 ) |
| A26T/T47A/M56K/L63P/S72G/Q82R/L98Q/M99L/Y105L | 126 | 2432 ( 0.5 ) | 835548 ( 1.2 ) | 2767 ( 1.7 ) | 404 ( 2.9 ) |
| A26T/M55T/L63P/S72G/L98Q/M99L/Y105L | 127 | 3226 ( 0.7 ) | 1085961 ( 1.5 ) | 2090 ( 1.3 ) | 1413 ( 10.1 ) |
| L12H/I18V/A42T/M55T/N58D/L98R/Y105L/L106I/P121S | 128 | 1764 ( 0.4 ) | 896733 ( 1.3 ) | 733 ( 0.4 ) | 170 ( 1.2 ) |
| I18T/A26T/L63P/S72G/L98Q/Y105L | 129 | 3265 ( 0.7 ) | 769820 ( 1.1 ) | 802 ( 0.5 ) | 145 ( 1.0 ) |
| L12F/K30R/S72G/Q82R/L98Q/M99L/Y105L | 130 | 1208 ( 0.3 ) | 766257 ( 1.1 ) | 1747 ( 1.0 ) | 718 ( 5.1 ) |
| L12P/L63P/S72G/L98Q/M99L/Y105L/L106N/I117L | 131 | 987 ( 0.2 ) | 782940 ( 1.1 ) | 998 ( 0.6 ) | 623 ( 4.4 ) |
| G29W/M87K/I93V/L98Q/M99L/Y105L | 132 | 2019 ( 0.4 ) | 767081 ( 1.1 ) | 7975 ( 4.8 ) | 786 ( 5.6 ) |
| P28L/E33V/L63P/S72G/L98Q/M99L/Y105L | 133 | 1163 ( 0.2 ) | 798068 ( 1.1 ) | 1849 ( 1.1 ) | 1161 ( 8.3 ) |
| G29W/T53S/M56K/L63P/Q82H/L98Q/M99I/Y105L | 134 | 4087 ( 0.9 ) | 425068 ( 0.6 ) | 5654 ( 3.4 ) | 956 ( 6.8 ) |
| I18F/L63P/L98Q/M99L/Y105L/P121S | 135 | 2392 ( 0.5 ) | 486401 ( 0.7 ) | 1765 ( 1.1 ) | 737 ( 5.3 ) |
| L63P/L98Q/M99L/Y105L/I108V | 136 | 3455 ( 0.7 ) | 730161 ( 1.0 ) | 2074 ( 1.2 ) | 592 ( 4.2 ) |
| A26T/A42V/Q45H/I67N/M87K/E97Q/M99L | 137 | 10573 ( 2.2 ) | 610530 ( 0.9 ) | 24030 ( 14.4 ) | 1282 ( 9.1 ) |
| E33M/L63P/S72G/L98Q/Y105L | 84 | 1984 ( 0.4 ) | 933740 ( 1.3 ) | 2401 ( 1.4 ) | 1849 ( 13.2 ) |
| M56V/E59G/L63P/S72G/M87K/I93V/L98Q/M99L/Y105L/I117E | 138 | 1940 ( 0.4 ) | 758136 ( 1.1 ) | 1552 ( 0.9 ) | 332 ( 2.4 ) |
| G29W/M87K/T89S/L98Q/M99L/Y105L/I108V/I117L | 139 | 3525 ( 0.7 ) | 913043 ( 1.3 ) | 9533 ( 5.7 ) | 232 ( 1.7 ) |

| Table E3. Binding and costimulatory bioactivity of variant CTLA-4-Fc polypeptides | | | | | |
|---|---|---|---|---|---|
| | SEQ ID NO (ECD) | Binding | | | MLR |
| Mutations | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | IFN-γ [pg/mL] ( Δ WT ) |
| L12P/M56V/L63P/V96I/L98Q/M99L/Y105L/Y115H | 140 | 1647 ( 0.3 ) | 891092 ( 1.3 ) | 1059 ( 0.6 ) | 907 ( 6.5 ) |
| G29W/T53S/M56K/T61N/L63P/L98Q/Y105L | 141 | 3375 ( 0.7 ) | 919607 ( 1.3 ) | 1454 ( 0.9 ) | 0 ( 0.0 ) |
| I18T/A26S/M55T/M56V/L63P/S72G/L98Q/M99L/Y105L/I117K | 142 | 2455 ( 0.5 ) | 782684 ( 1.1 ) | 1686 ( 1.0 ) | 530 ( 3.8 ) |
| I18T/T61R/L63P/S72G/L98Q/M99L/Y105L | 143 | 3315 ( 0.7 ) | 926617 ( 1.3 ) | 2390 ( 1.4 ) | 296 ( 2.1 ) |
| L12P/L63P/S72G/L98Q/M99L/Y105L | 144 | 1784 ( 0.4 ) | 1045369 ( 1.5 ) | 1510 ( 0.9 ) | 968 ( 6.9 ) |
| E33M/L63P/S72G/L98Q/Y105L/I108F | 145 | 1481 ( 0.3 ) | 820016 ( 1.2 ) | 2109 ( 1.3 ) | 766 ( 5.5 ) |
| L12P/R16H/A26T/T61S/L63P/M87V/L98Q/M99L/Y105L/L106I/I117L | 146 | 1926 ( 0.4 ) | 895016 ( 1.3 ) | 1046 ( 0.6 ) | 593 ( 4.2 ) |
| G29W/T53S/M56K/N58S/L63P/M87V/L98Q/Y105L/P121S | 147 | 7819 ( 1.6 ) | 778254 ( 1.1 ) | 2249 ( 1.4 ) | 0 ( 0.0 ) |
| G29W/L63P/S72G/L98Q/Y105L/P121S | 148 | 3395 ( 0.7 ) | 763120 ( 1.1 ) | 1559 ( 0.9 ) | 0 ( 0.0 ) |
| G29W/T53S/M56K/N58S/L63P/M87V/L98Q/Y105L | 149 | 8116 ( 1.7 ) | 257214 ( 0.4 ) | 2517 ( 1.5 ) | 0 ( 0.0 ) |
| G29W/T53S/M56K/N58S/L63P/M87V/L98Q/Y105L/I108V | 150 | 7775 ( 1.6 ) | 271930 ( 0.4 ) | 3703 ( 2.2 ) | 45 ( 0.3 ) |
| G29W/T53S/L63P/S72G/L98Q/Y105L | 151 | 4497 ( 0.9 ) | 174601 ( 0.2 ) | 1545 ( 0.9 ) | 0 ( 0.0 ) |
| V10A/G29W/T53S/M56K/L63P/L98Q/Y105L/P121S | 152 | 6058 ( 1.3 ) | 766570 ( 1.1 ) | 1612 ( 1.0 ) | 0 ( 0.0 ) |
| WT CTLA-4 | 1 | 4775 ( 1.0 ) | 708753 ( 1.0 ) | 1664 ( 1.0 ) | 140 ( 1.0 ) |

**Example 2. Generation and Assay of CTLA-4 Consensus Variants**

**[0638]** Additional variants of CTLA-4 ECD were designed by identifying consensus residues identified in the screen described in Example 1 that were commonly associated with variants that exhibited improved CD80, CD86, and/or ICOSL binding and/or demonstrated suppression of interferon-gamma secretion in the MLR assay. The selected consensus mutations included I18T, A26T, E33V,

T53S, M55T, M56K, N58S, L63P, M87V, L98Q, M99L, and Y105L. The consensus mutants were used to generate variant CTLA-4 ECDs by site-directed mutagenesis with reference to the wild-type sequence set forth in SEQ ID NO:1, which was then formatted as an Fc fusion protein as described in Example 1. The variant CTLA-4 ECD-Fc fusions were tested for binding and bioactivity as described below.

### *A. Binding and Bioactivity*

#### *1. Binding to Cell-Expressed Counter Structures*

**[0639]** To produce cells expressing cognate binding partners, full-length mammalian surface expression constructs for each of human CD80, CD86, and ICOSL were designed in pcDNA3.1 expression vector (Life Technologies) and sourced from Genscript, USA. Binding studies were carried out using the Expi293F transient transfection system (Life Technologies, USA) described above. The number of cells needed for the experiment was determined, and the appropriate 30 mL scale of transfection was performed using the manufacturer's suggested protocol. For each counter structure or mock 30 mL transfection, 75 million Expi293F cells were incubated with 30 μg expression construct DNA and 1.5 mL diluted ExpiFectamine™ 293 reagent for 48 hours, at which point cells were harvested for staining.

**[0640]** In some instances, cells with stable expression of cognate binding partners were used. Chinese hamster ovarian cells (CHO) were stably transduced by lentivirus for surface expression of full-length human CD80, CD86, or ICOSL.

**[0641]** For flow cytometric analysis, 200,000 cells of a given transient transfection, stable cell line, or appropriate negative control were plated in 96 well round bottom plates. Cells were spun down and suspended in staining buffer (PBS (phosphate buffered saline), 1% BSA (bovine serum albumin), and 0.1% sodium azide) for 20 minutes to block non-specific binding. Afterwards, cells were centrifuged again and suspended in staining buffer containing 100 nM to 100 pM CTLA-4 variant Fc fusion protein or control in 50 μL. Primary staining was performed for 45 minutes, before washing cells in staining buffer twice. Bound CTLA-4 was detected with PE-conjugated anti-human IgG (Jackson ImmunoResearch, USA) diluted 1:150 in 50 μL staining buffer and incubated for 30 minutes. Alternatively, bound CTLA-4 was detected with anti-CTLA-4 antibody (Biolegend, USA) diluted 1:130 in 50 μL staining buffer for 30 minutes, before washing cells in staining buffer twice. Anti-CTLA-4 antibody was then detected with PE-conjugated anti-mouse IgG (Jackson ImmunoResearch, USA) diluted 1:150 in 50 μL staining buffer and incubated for 30 minutes.

**[0642]** After final incubation, cells were washed twice to remove unbound conjugated antibodies, fixed in 2% formaldehyde/PBS, and analyzed on a Hypercyt (Intellicyte, USA) or LSRII (Becton Dickinson, USA) flow cytometer.

**[0643]** Mean Fluorescence Intensity (MFI) was calculated for each sample with Cell Quest Pro software (Becton Dickinson, USA), FlowJo software (FlowJo, USA), or Forcyte software (Intellicyt, USA).

a. CD86 Blockade Bioassay

**[0644]** Select CTLA-4 variant Fc fusion proteins were assayed for capacity to block CD86-CD28 mediated costimulation as determined by a CD86 blockade bioassay. Artificial antigen presenting cells (APCs) were generated by transducing K562 cells with lentivirus to express cell surface anti-human CD3 single chain Fv (OKT3) and human CD86, yielding K562/OKT3/CD86. Effector cells were generated by transducing Jurkat cells expressing an IL-2-luciferace reporter (Promega) with lentivirus to express a chimeric receptor composed of the extracellular domain of human ICOS and the intracellular domain of human CD28, yielding Jurkat/IL-2/ICOS-CD28. APCs were plated in 33 μL/well of assay buffer (RPMI1640 with 5% FBS) at $2x10^4$ cells/well with CTLA-4-Fc or control proteins in 33 μL/well at 300 nM. APCs and proteins were incubated for 20 minutes at room temperature before the addition of effector cells at $2x10^5$ cell/well in 33 μL/well. The plates were transferred to a 37 °Celsius incubation chamber, humidified with 5% CO2 for 5 hours, then removed and allowed to acclimate to room temperature for 15 minutes. 100 μL/well of cell lysis and luciferase substrate solution (BioGlo™ luciferase reagent, Promega) was added to each plate and incubated on an orbital shaker for 10 minutes. Relative luminescence values (RLU) were determined for each test sample by measuring luminescence with a 1 second per well integration time using a Cytation 3 imaging reader (BioTek instruments). The percent inhibition mediated by CD86 blockade was determined using the following formula: [(Avg. Control RLU – Experimental RLU)/(Avg. Control RLU)]x100.

### *B. Results*

**[0645]** The results are summarized below in Table E4. The values for binding CD80, CD86, and ICOSL (MFI) and percent inhibition CD28 costimulation are provided in addition to the relative ratio, as compared to the corresponding binding and CD86 blockade of the unmodified CTLA-4 polypeptide (ΔWT) for each experiment. As indicated, certain mutations and combinations of mutations were associated with a substantial increase in binding of CTLA-4 ECD to ICOSL, independent of the change in binding to either CD80 or CD86. In some cases, increases in binding to one or both of CD80 or CD86 also were observed.

| Table E4. Binding and bioactivity of consensus variant CTLA-4-Fc polypeptides | | | | | |
|---|---|---|---|---|---|
| | SEQ ID NO (ECD) | Binding | | | CD86 Blockade Bioassay ( Δ WT ) |
| Mutations | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | |
| T53S, M56K, N58S, L63P, M87V, L98Q, Y105L | 162 | 631192 (1.2) | 497901 (0.9) | 215054 (50.9) | 88.2 (1.3) |
| I18T, A26T, M55T, M56K, L63P, L98Q, M99L, Y105L | 163 | 759480 (1.4) | 657099 (1.1) | 89672 (21.2) | 40.2 (0.6) |
| I18T, A26T, M56K, L63P, L98Q, Y105L | 164 | 496119 (0.9) | 601631 (1.0) | 295395 (69.9) | 86.1 (1.2) |
| T53S, L63P, L98Q | 165 | 564111 (1.1) | 571155 (1.0) | 11541 (2.7) | 86.2 (1.2) |
| T53S, L63P, Y105L | 166 | 526605 (1.0) | 568901 (1.0) | 20739 (4.9) | 86.4 (1.2) |
| T53S, M56K, N58S, L63P, M87V, Y105L | 168 | 610377 (1.2) | 604604 (1.0) | 48034 (11.4) | 86.7 (1.3) |
| L98Q, M99L, Y105L | 59 | 875290 (1.7) | 686788 (1.2) | 116699 (27.6) | 33.9 (0.5) |
| E33V, L98Q, Y105L | 174 | 811261 (1.5) | 580048 (1.0) | 101877 (24.1) | 32.5 (0.5) |
| E33V, M99L | 177 | 758165 (1.4) | 618183 (1.1) | 71903 (17.0) | 85.2 (1.2) |
| T53S, M56K, N58S, L63P, M87V, L98Q | 167 | 347188 (0.7) | 555921 (1.0) | 7241 (1.7) | 82.6 (1.2) |
| T53S, M56K, N58S, L63P, L98Q, Y105L | 169 | 795550 (1.5) | 557059 (1.0) | 248668 (58.8) | 87.4 (1.3) |
| T53S, M56K, N58S, M87V, L98Q, Y105L | 170 | 1133587 (2.1) | 676071 (1.2) | 35087 (8.3) | 88.7 (1.3) |
| T53S, M56K, L63P, M87V, L98Q, Y105L | 171 | 736640 (1.4) | 546545 (0.9) | 234716 (55.5) | 90.1 (1.3) |
| T53S, N58S, L63P, M87V, L98Q, Y105L | 172 | 637509 (1.2) | 508878 (0.9) | 108784 (25.7) | 86.8 (1.3) |
| M56K, N58S, L63P, M87V, L98Q, Y105L | 173 | 688049 (1.3) | 574298 (1.0) | 258574 (61.2) | 85.9 (1.2) |
| E33V, L98Q, M99L, | 176 | 975697 (1.8) | 628740 (1.1) | 137713 (32.6) | 14.1 (0.2) |
| Wild-type | 1 | 529140 (1.0) | 579615 (1.0) | 4228 (1.0) | 69.1 (1.0) |

**Example 3. Generation and Assay of Select CTLA-4 Variants**

**[0646]** A further panel of CTLA-4 ECD variants was designed with mutations from a variant CTLA-4 identified in the screen described in Example 1, specifically the variant set forth in SEQ ID NO: 113 containing mutations L12F/R16H/G29W/M56T/L98Q/Y105L, which was associated with enhanced binding to CD80, CD86, and ICOSL and suppression of interferon-gamma. In some cases, S72G was

included because it had been identified as a hot spot that had occurred in greater than 35% of the other top 50 hits that were identified as having suppressive activity. For some generated variants, the strategy included removal of some mutations (reversion mutations), for example, to reduce the number of mutations in the variant. Variant CTLA-4 ECDs were generated by site-directed mutagenesis with reference to the wild-type sequence set forth in SEQ ID NO:1, which was then formatted as an Fc fusion protein as described in Example 1. The variant CTLA-4 ECD-Fc fusions were tested for binding and bioactivity as described in Example 2.

**[0647]** **Table E5** provides the values for binding CD80, CD86, and ICOSL (MFI) and percent inhibition CD28 costimulation in addition to the relative ratio, as compared to the corresponding binding and CD86 blockade of the unmodified CTLA-4 polypeptide (ΔWT) for each experiment.

**Table E5. Binding and bioactivity of reversion variant CTLA-4-Fc polypeptides**

| Mutations | SEQ ID NO (ECD) | Binding | | | MLR |
|---|---|---|---|---|---|
| | | CD80 MFI ( Δ WT ) | CD86 MFI ( Δ WT ) | ICOSL MFI ( Δ WT ) | CD86 Blockade Bioassay ( Δ WT ) |
| L12F, R16H, G29W, M56T, L98Q | 178 | 76155 (1.5) | 86548 (1.2) | 959 (0.8) | 72.3 (0.9) |
| L12F, R16H, G29W, M56T, Y105L | 179 | 73996 (1.4) | 72293 (1.0) | 1944 (1.7) | 77.8 (1.0) |
| L12F, R16H, G29W, L98Q, Y105L | 180 | 60527 (1.2) | 78181 (1.1) | 862 (0.7) | 89.0 (1.1) |
| L12F, R16H, M56T, L98Q, Y105L | 181 | 70120 (1.4) | 70437 (1.0) | 1265 (1.1) | 86.8 (1.1) |
| G29W, M56T, L98Q, Y105L | 182 | 70579 (1.4) | 65251 (0.9) | 612 (0.5) | 88.6 (1.1) |
| L12F, G29W, L98Q, Y105L | 183 | 66677 (1.3) | 85018 (1.2) | 807 (0.7) | 90.0 (1.1) |
| L12F, L98Q, Y105L | 184 | 67142 (1.3) | 85125 (1.2) | 2584 (2.2) | 86.9 (1.1) |
| R16H, L98Q, Y105L | 185 | 67259 (1.3) | 70269 (1.0) | 1018 (0.9) | 89.8 (1.1) |
| G29W, L98Q, Y105L | 186 | 90170 (1.8) | 64097 (0.9) | 570 (0.5) | 90.0 (1.1) |
| M56T, L98Q, Y105L | 187 | 68644 (1.3) | 70222 (1.0) | 700 (0.6) | 88.0 (1.1) |
| L12F, R16H, G29W, M56T, S72G, L98Q, Y105L | 188 | 46175 (0.9) | 58464 (0.8) | 613 (0.5) | 88.3 (1.1) |
| G29W, M56T, S72G, L98Q, Y105L | 189 | 55706 (1.1) | 67962 (0.9) | 534 (0.5) | 88.6 (1.1) |
| Wild-type | 1 | 51269 (1.0) | 73502 (1.0) | 1160 (1.0) | 80.5 (1.0) |

**Example 4. Identification of Affinity Modified TACI Extracellular Domain Polypeptide as a B cell Inhibitory Molecule (BIM)**

**[0648]** This examples describes exemplary TACI polypeptide B cell inhibitory molecules (BIMs) that are employed as part of a provided multi-domain immunomodulatory protein with a T cell inhibitory molecule (TIM), including methods for engineering and identifying affinity-modified (variant) TACI polypeptides that bind (e.g. increased compared to wild-type) to ligands of a B cell stimulatory receptor.

**[0649]** This Example describes the generation of mutant DNA constructs of human TACI TNFR domains (TD) for translation and expression on the surface of yeast as yeast display libraries, introduction of DNA libraries into yeast, and selection of yeast cells expressing affinity-modified variants of the extracellular domain (ECD) of TACI containing at least one TD (TACI vTD).

**A. Generation of Mutant DNA constructs of TACI TNFR Domains**

**[0650]** Libraries containing random substitutions of amino acids were constructed to identify variants of the extracellular domain (ECD) of TACI. Constructs were generated based on a wildtype human TACI sequence containing an ECD portion of TACI that included either (1) both cysteine-rich protein domains (CRD, CRD1/CRD2) as set forth in SEQ ID NO: 516 (corresponding to residues 29-110 as set forth in UniProt Accession No. O14836), or (2) only a single CRD (CRD2) as set forth in SEQ ID NO: 528 (corresponding to residues 68-110 as set forth in UniProt Accession No. O14836).

TACI ECD (29-110) (SEQ ID NO: 516):
VAMRSCPEEQYWDPLLGTCMSCKTICNHQSQRTCAAFCRSLSCRKEQGKFYDHLLRDCISCA
SICGQHPKQCAYFCENKLRS

TACI ECD (68-110) (SEQ ID NO: 528):
SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRS

**[0651]** DNA encoding the wild-type TACI ECD domain was cloned between the BamHI and KpnI sites of the modified yeast expression vector PBYDS03 (Life Technologies USA) which placed the TACI ECD N-terminal to the yeast surface anchoring domain Sag1 (the C-terminal domain of yeast α-agglutinin) with an in-frame HA fusion tag N-terminal to the TACI ECD sequence and a c-Myc fusion tag C-terminal to the TACI ECD sequence. Expression in this vector is controlled through the inducible GAL1 promoter. After verification of the correct DNA sequence, the wild-type TACI ECD DNA construct was used as template for error-prone PCR to introduce random mutations across the TACI ECD sequence at a frequency of 2-5 mutations per gene copy. The Genemorph II Kit (Agilent, USA) was used in combination with titrating amounts of MnCl2 from 0.0 to 0.6 mM to achieve the desired error rate. After error-prone PCR, the mutagenized DNA was gel purified using the NucleoSpin® Gel and PCR

Clean-up kit (Macherey-Nagel, Germany). This isolated DNA fragment was then PCR amplified with OneTaq 2x PCR master mix (New England Biolabs, USA) using primers containing 48 bp overlap regions homologous to pBYDS03 for preparation for large scale yeast electroporation. The TACI ECD DNA insert was gel-purified and resuspended in sterile, deionized water at a nominal concentration of 500 ng/µL.

**[0652]** To prepare the vector for transformation, pBYDS03 was digested with BamHI-HF and KpnI-HF restriction enzymes (New England Biolabs, USA) and the large vector fragment (expected size: 7671 bp) was gel-purified and dissolved in sterile, deionized water at a nominal concentration of 500 ng/µL. To prepare for yeast transformation, 12 µg of library DNA insert was mixed with 4 µg of linearized vector for each electroporation.

**[0653]** To introduce random DNA libraries into yeast, the Saccharomyces cerevisiae strain BJ5464 (ATCC.org; ATCC number 208288) was prepared immediately prior to electroporation as detailed in Benatuil, L. et.al., Protein Eng Des Sel. 2010 Apr;23(4):155-159. Briefly, an overnight stationary-phase culture of BJ5464 was passaged to $OD_{600}$ 0.3 in 100 mL YPD medium (10 g/L yeast nitrogen base, 20 g/L Peptone and 20 g/L D-(+)-Glucose) and placed in a platform shaker at 30 °C and 300 rpm until the inoculated cultures reached $OD_{600}$ 1.6. After ~5 hours, cells were harvested by centrifugation and kept on ice for the remainder of the protocol unless otherwise stated. After harvesting, cells were washed twice with 50 mL ice-cold water and once with electroporation buffer (1 M Sorbitol, 1 mM CaCl2). Collected cells were conditioned by re-suspending in 20 mL 0.1 M LiAc/10 mM DTT and shaking at 225 rpm in a culture flask for 30 minutes at 30 °C. Conditioned cells were immediately centrifuged, washed twice with electroporation buffer, and resuspended with ~100-200 µl of electroporation buffer to bring the volume to 1 mL. This conditioned cell suspension was sufficient for two electroporation reactions in 400 µl cuvettes.

**[0654]** For each electroporation, 12 µg of library DNA insert and 4 µg of linearized pBYDS03 vector (described above) was mixed with 400 µl of electrocompetent BJ5464 and transferred to a pre-chilled BioRad GenePulser cuvette with 2 mm electrode gap. The mixtures were kept on ice for 5 minutes, prior to electroporation using a BTX ECM399 exponential decay wave electroporation system at 2500V. Immediately following electroporation, cells were added to 8 mL of 1:1 mixture of 1 M Sorbitol:1X YPD, and left at room temperature without shaking for 10 min, then placed on platform shaker for 1 hr at 225 rpm and 30 °C. Cells were collected by centrifugation and resuspended in 250 mL SCD-Leu medium to accommodate the LEU2 selective marker carried by modified plasmid pBYDS03. One liter of SCD-Leu media was generated with 14.7 gm sodium citrate, 4.29 gm citric acid monohydrate, 20 gm dextrose, 6.7 gm yeast nitrogen base, and 1.6 gm yeast synthetic drop-out media supplement without leucine. The medium was filter sterilized before use using a 0.22 µm vacuum filter device. Library size was estimated by spotting serial dilutions of freshly recovered cells on an SCD-Leu

agar plate in the dilution range of $10^{-5}$ to $10^{-10}$ and extrapolating by counting colonies after three days. The remainder of the electroporated culture was grown to saturation and cells from this culture were subcultured 1/100 into the same medium once more and grown to saturation to minimize the fraction of untransformed cells and to allow for segregation of plasmid from cells that may contain two or more library variants. To maintain library diversity, this subculturing step was carried out using an inoculum that contained at least 10x more cells than the calculated library size. Cells from the second saturated culture were resuspended in fresh medium containing sterile 25% (weight/volume) glycerol to a density of 1 x $10^{10}$/mL and frozen and stored at -80ºC (frozen library stock).

**[0655]** A number of cells equal to at least 10 times the estimated library size were thawed from individual library stocks, suspended to 0.5 x $10^7$ cells/mL in non-inducing SCD-Leu medium, and grown overnight. The next day, a number of cells equal to 10 times the library size were centrifuged at 2000 RPM for two minutes and resuspended to 0.5 x $10^7$ cells/mL in inducing SCDG-Leu media. One liter of SCDG-Leu induction media was generated with 5.4 gm $Na_2HPO_4$, 8.56 gm $NaH_2PO_4 \bullet H_20$, 20 gm galactose, 2.0 gm dextrose, 6.7 gm yeast nitrogen base, and 1.6 gm yeast synthetic drop out media supplement without leucine dissolved in water and sterilized through a 0.22 µm membrane filter device. The culture was grown in induction medium overnight at 30 °C to induce expression of library proteins on the yeast cell surface.

**[0656]** Following overnight induction of the TACI ECD libraries, a number of cells equivalent to 10 times the estimated library diversity were sorted by magnetic separation using Dynabeads™ His-Tag magnetic beads preloaded with BAFF-9xHis to enrich for TACI ECD variants with the ability to bind their exogenous recombinant counter-structure proteins. The outputs from the magnetic separation were used in a subsequent FACS selection scheme involving four rounds of positive selections alternating between BAFF-9xHis and APRIL-FLAG, with simultaneous 10-fold reduction in counter structure concentration each round (e.g., FACS1: 50 nM APRIL-FLAG; FACS4: 0.05 nM BAFF-9xHis). The incubation volume was adjusted to maintain at least a 10-fold stoichiometric excess of counter structure over the total number of yeast-displayed TACI ECD variant molecules (assuming 100,000 copies of protein per cell) to avoid ligand depletion artifacts which can reduce library discrimination. Binding of BAFF-9xHis and APRIL-FLAG to TACI ECD variants was detected with PE conjugated anti-6xHis tag antibody (BioLegend, USA) and PE conjugated anti-FLAG-tag antibody, respectively. Variants from FACS3 and FACS4 outputs were isolated for DNA sequencing and subsequent cloning for recombinant Fc fusion expression.

**[0657]** A second cycle of random mutagenesis was carried out on yeast cell outputs from the FACS4 BAFF-9xHis selections described above. The positive selection protocol with alternating counter structures per sort was the same as the first cycle except that the order of counter structures was switched

(e.g., FACS1: 50 nM BAFF-9xHis; FACS4: 0.05 nM APRIL-FLAG). Additional variants were chosen from FACS3 and FACS4 yeast cell outputs.

**[0658]** TACI ECD variant inserts from FACS3 and FACS4 outputs from both cycle 1 and cycle 2 selections, as described above, were subcloned into an Fc fusion vector for sequence analysis of individual clones.

**[0659]** Output cell pools from selected TACI ECD FACS sorts were grown to terminal density in SCD-Leu selection medium and plasmid DNA was isolated using a yeast plasmid DNA isolation kit (Zymoresearch, USA). For generation of Fc fusions, the affinity matured TACI ECD variants were PCR amplified with primers containing 40 bp homologous regions on either end with an AfeI and BamHI digested Fc fusion vector encoding and in-frame with the Fc region to carry out in vitro recombination using Gibson Assembly Master Mix (New England Biolabs). The Gibson Assembly reaction was added to the E. coli strain NEB5alpha (New England Biolabs, USA) for heat shock transformation following the manufacturer's instructions.

**[0660]** Dilutions of transformation reactions were plated onto LB-agar containing 100 μg/mL carbenicillin (Teknova, USA) to isolate single colonies for selection. Generally, up to 96 colonies from each transformation were then grown in 96 well plates to saturation overnight at 37°C in LB-broth containing 100 μg/mL carbenicillin (Teknova cat # L8112) and a small aliquot from each well was submitted for DNA sequencing to identify mutation(s) in all clones.

**[0661]** After sequence analysis and identification of clones of interest, plasmid DNA was prepared using the MidiPlus kit (Qiagen).

**[0662]** Recombinant variant Fc fusion proteins were produced from suspension-adapted human embryonic kidney (HEK) 293 cells using the Expi293 expression system (Invitrogen, USA). Supernatant was harvested and the Fc protein was captured on Mab SelectSure (GE Healthcare cat. no. 17543801). Protein was eluted from the column using 50mM Acetate pH3.6. The MabSelect Sure eluate was pooled and the pH was adjusted to above pH5.0. This material was then polished on a Preparative SEC column, to generate highly purified monomeric material. This material was buffer exchanged into 10mM Acetate, 9% Sucrose pH 5.0. The protein purity was assessed by analytic SEC. Material was vialed and stored at -80.

**[0663]** Amino acid substitutions in selected TACI vTDs that were identified and generated by the selection are set forth in Table 2. Selected vTDs were tested for binding and functional activity as described in Example 6.

**Example 5. Identification of Affinity Modified BCMA Extracellular Domain Polypeptide and Immunomodulatory Proteins**

**[0664]** This examples describes exemplary BCMA polypeptide B cell inhibitory molecules (BIMs) that are employed as part of a provided multi-domain immunomodulatory protein with a T cell inhibitory molecule (TIM), including methods for engineering and identifying affinity-modified (variant) BCMA polypeptides that bind (e.g. increased compared to wild-type) to ligands of a B cell stimulatory receptor. The variant BCMA extracellular polypeptides also were formatted as an immunomodulatory protein as a BCMA Fc-fusion protein without a T cell inhibitory molecule.

**[0665]** This Example describes the generation of mutant DNA constructs of human BCMA TNFR domains (TD) for translation and expression on the surface of yeast as yeast display libraries, introduction of DNA libraries into yeast, and selection of yeast cells expressing affinity-modified variants of the extracellular domain (ECD) of BCMA containing at least one TD (BCMA vTD). The selected BCMA vTD were then formatted as Fc fusion proteins

**A. Generation of Mutant DNA constructs of BCMA Domains**

**[0666]** Libraries containing random substitutions of amino acids were constructed to identify variants of the extracellular domain (ECD) of BCMA. Constructs were generated based on a wildtype human BCMA sequence containing an ECD portion of BCMA that included the cysteine-rich protein domain (CRD) as set forth in SEQ ID NO: 356 (corresponding to residues 2-54 as set forth in UniProt Accession No.Q02223 designated "BCMA ECD (2-54)" as follows:

**[0667]** BCMA ECD (2-54) (SEQ ID NO: 356):
LQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVKGTNA

**[0668]** DNA encoding the wild-type BCMA ECD domain was cloned between the BamHI and KpnI sites of the modified yeast expression vector PBYDS03 (Life Technologies USA) which placed the BCMA ECD N-terminal to the yeast surface anchoring domain Sag1 (the C-terminal domain of yeast α-agglutinin) with an in-frame HA fusion tag N-terminal to the BCMA ECD sequence and a c-Myc fusion tag C-terminal to the BCMA ECD sequence. Expression in this vector is controlled through the inducible GAL1 promoter. After verification of the correct DNA sequence, the wild-type BCMA ECD DNA construct was used as template for error-prone PCR to introduce random mutations across the BCMA ECD sequence at a frequency of 2-5 mutations per gene copy. The Genemorph II Kit (Agilent, USA) was used in combination with titrating amounts of MnCl2 from 0.0 to 0.6 mM to achieve the desired error rate. After error-prone PCR, the mutagenized DNA was gel purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Germany). This isolated DNA fragment was then PCR amplified with OneTaq 2x PCR master mix (New England Biolabs, USA) using primers containing 48 bp

overlap regions homologous to pBYDS03 for preparation for large scale yeast electroporation. The BCMA ECD DNA insert was gel-purified and resuspended in sterile, deionized water at a nominal concentration of 500 ng/µL.

**[0669]** To prepare the vector for transformation, pBYDS03 was digested with BamHI-HF and KpnI-HF restriction enzymes (New England Biolabs, USA) and the large vector fragment (expected size: 7671 bp) was gel-purified and dissolved in sterile, deionized water at a nominal concentration of 500 ng/µL. To prepare for yeast transformation, 12 µg of library DNA insert was mixed with 4 µg of linearized vector for each electroporation.

**[0670]** To introduce random DNA libraries into yeast, the Saccharomyces cerevisiae strain BJ5464 (ATCC.org; ATCC number 208288) was prepared immediately prior to electroporation as detailed in Benatuil, L. et.al., Protein Eng Des Sel. 2010 Apr;23(4):155-159. Briefly, an overnight stationary-phase culture of BJ5464 was passaged to OD600 0.3 in 100 mL YPD medium (10 g/L yeast nitrogen base, 20 g/L Peptone and 20 g/L D-(+)-Glucose) and placed in a platform shaker at 30 °C and 300 rpm until the inoculated cultures reached OD600 1.6. After ~5 hours, cells were harvested by centrifugation and kept on ice for the remainder of the protocol unless otherwise stated. After harvesting, cells were washed twice with 50 mL ice-cold water and once with electroporation buffer (1 M Sorbitol, 1 mM CaCl2). Collected cells were conditioned by re-suspending in 20 mL 0.1 M LiAc/10 mM DTT and shaking at 225 rpm in a culture flask for 30 minutes at 30 °C. Conditioned cells were immediately centrifuged, washed twice with electroporation buffer, and resuspended with ~100-200 µl of electroporation buffer to bring the volume to 1 mL. This conditioned cell suspension was sufficient for two electroporation reactions in 400 µl cuvettes.

**[0671]** For each electroporation, 12 µg of library DNA insert and 4 µg of linearized pBYDS03 vector (described above) was mixed with 400 µl of electrocompetent BJ5464 and transferred to a pre-chilled BioRad GenePulser cuvette with 2 mm electrode gap. The mixtures were kept on ice for 5 minutes, prior to electroporation using a BTX ECM399 exponential decay wave electroporation system at 2500V. Immediately following electroporation, cells were added to 8 mL of 1:1 mixture of 1 M Sorbitol:1X YPD, and left at room temperature without shaking for 10 min, then placed on platform shaker for 1 hr at 225 rpm and 30 °C. Cells were collected by centrifugation and resuspended in 250 mL SCD-Leu medium to accommodate the LEU2 selective marker carried by modified plasmid pBYDS03. One liter of SCD-Leu media was generated with 14.7 gm sodium citrate, 4.29 gm citric acid monohydrate, 20 gm dextrose, 6.7 gm yeast nitrogen base, and 1.6 gm yeast synthetic drop-out media supplement without leucine. The medium was filter sterilized before use using a 0.22 µm vacuum filter device. Library size was estimated by spotting serial dilutions of freshly recovered cells on an SCD-Leu agar plate in the dilution range of 10-5 to 10-10 and extrapolating by counting colonies after three days. The remainder of the electroporated culture was grown to saturation and cells from this culture were

subcultured 1/100 into the same medium once more and grown to saturation to minimize the fraction of untransformed cells and to allow for segregation of plasmid from cells that may contain two or more library variants. To maintain library diversity, this subculturing step was carried out using an inoculum that contained at least 10x more cells than the calculated library size. Cells from the second saturated culture were resuspended in fresh medium containing sterile 25% (weight/volume) glycerol to a density of 1 x $10^{10}$/mL and frozen and stored at -80ºC (frozen library stock).

**[0672]** A number of cells equal to at least 10 times the estimated library size were thawed from individual library stocks, suspended to 0.5 x $10^7$ cells/mL in non-inducing SCD-Leu medium, and grown overnight. The next day, a number of cells equal to 10 times the library size were centrifuged at 2000 RPM for two minutes and resuspended to 0.5 x $10^7$ cells/mL in inducing SCDG-Leu media. One liter of SCDG-Leu induction was generated with 5.4 gm Na2HPO4, 8.56 gm NaH2PO4•H20, 20 gm galactose, 2.0 gm dextrose, 6.7 gm yeast nitrogen base, and 1.6 gm yeast synthetic drop out media supplement without leucine dissolved in water and sterilized through a 0.22 μm membrane filter device. The culture was grown in induction medium overnight at 30 °C to induce expression of library proteins on the yeast cell surface.

**[0673]** Following overnight induction of the naïve BCMA ECD libraries, a number of cells equivalent to 10 times the estimated library diversity were sorted by magnetic separation using Dynabeads™ His-Tag magnetic beads preloaded with BAFF-9xHis to enrich for BCMA ECD variants with the ability to bind their exogenous recombinant counter-structure proteins. The outputs from the magnetic separation were used in a subsequent FACS selection scheme involving four rounds of positive selections alternating between BAFF-9xHis and APRIL-FLAG, with simultaneous 10-fold reduction in counter structure concentration each round (e.g., FACS1: 50 nM APRIL-FLAG; FACS4: 0.05 nM BAFF-9xHis). The incubation volume was adjusted to maintain at least a 10-fold stoichiometric excess of counter structure over the total number of yeast-displayed BCMA ECD variant molecules (assuming 100,000 copies of protein per cell) to avoid ligand depletion artifacts which can reduce library discrimination. Binding of BAFF-9xHis and APRIL-FLAG to BCMA ECD variants was detected with PE conjugated anti-6xHis tag antibody (BioLegend, USA) and PE conjugated anti-FLAG-tag antibody, respectively. Variants from FACS3 and FACS4 outputs were isolated for DNA sequencing and subsequent cloning for recombinant Fc fusion expression.

**[0674]** A second cycle of random mutagenesis was carried out on yeast cell outputs from the FACS4 BAFF-9xHis selections described above. The positive selection protocol with alternating counter structures per sort was the same as the first cycle except that the order of counter structures was switched (e.g., FACS1: 50 nM BAFF-9xHis; FACS4: 0.05 nM APRIL-FLAG). Additional variants were chosen from FACS3 and FACS4 yeast cell outputs.

### *A. Generation of Degenerate Codon Mutant DNA Constructs of BCMA TNFR Domains*

**[0675]** This Example describes the design of targeted degenerate codon DNA libraries of human BCMA TNFR domains for translation and expression on the surface of yeast as yeast display libraries, introduction of DNA libraries into yeast, and selection of yeast cells expressing affinity-modified variants of BCMA TNFR.

**[0676]** Targeted DNA oligonucleotide libraries were constructed based on available crystal structure and functional data following in vitro characterization of the recombinant BCMA ECD variant Fc fusion proteins isolated from cycle 1 and cycle 2 random library affinity maturation selections. First, BCMA positions for targeted mutagenesis were restricted to BCMA:BAFF interfacial contacts (PDB ID: 1XU2), using a per-residue weighted average distance cutoff of 4.5 Angstroms (PyMOL, Schrödinger). Second, sequence-function heat maps were generated using available $IC_{50}$ data to identify positions where mutation generally improved binding to BAFF and APRIL. To aid visual inspection, a position-specific propensity score (PSPS) was calculated by comparing the frequency of all mutations in recombinant BCMA ECD variant Fc fusions having an $IC_{50}$ within the top 10% relative to whole-population mutational frequency. The PSPS was further scaled by the frequency of mutation within the top 10% (i.e., numerator term of PSPS) to help correct for biasing of positions with high mutational load. The average $IC_{50}$ for cycle 2 outputs was one order of magnitude lower for BAFF than APRIL, and thus, preference was given to positions where substitution generally improved BAFF binding.

**[0677]** Finally, consideration of recombinant BCMA ECD variant Fc protein expression yields and homogeneity evaluated from Protein A affinity chromatography and size exclusion chromatography, respectively. Taken together, seven positions were selected for design of three independent degenerate codon BCMA ECD libraries: H19, I22, Q25, S30, N31, L35, and T36. Site saturation mutagenesis at each of the desired positions indicated in Table E6 was carried out using the mixed base set “NNK”, which encodes all 20 proteinogenic amino acids. Each degenerate codon library was designed using SnapGene (GSL Biotech LLC, USA) and synthesized as a single-stranded, full-length DNA oligonucleotide Ultramer® from Integrated DNA Technologies.

Table E6. Targeted library positions selected for saturation mutagenesis “NKK”

| Position | 19 | 22 | 25 | 30 | 31 | 35 | 36 |
|---|---|---|---|---|---|---|---|
| Wt residue | H | I | Q | S | N | L | T |
| Design 1 | X | X | | X | X | X | |
| Design 2 | X | | X | | X | X | X |
| Design 3 | X | X | X | X | X | X | X |

**[0678]** Targeted NNK ssDNA Ultramers® were prepared and introduced into yeast essentially as described in Example 4. The libraries were used to select yeast expressing affinity modified variants of BCMA ECD substantially as described in Example 4. Selections were performed essentially as described in Example 4 following the cycle 1 random library selection protocol. Additional variants identified in the screen as described are set forth in Table E14.

***B. Reformatting Selection Outputs as Fc-Fusions***

**[0679]** BCMA ECD variant outputs from the selections, as described above, were subcloned into an Fc fusion vector for sequence analysis of individual clones To generate recombinant immunomodulatory proteins as Fc fusion proteins containing an ECD of BCMA with at least one affinity-modified domain (e.g., variant BCMA ECD-Fc), the encoding DNA was generated to encode a protein as follows: variant BCMA domain followed by a linker of 7 amino acids (GSGGGGS; SEQ ID NO: 590) followed by a human IgG1 effectorless Fc sequence containing the mutations L234A, L235E and G237A, by the Eu Index numbering system for immunoglobulin proteins. Since the construct does not include any antibody light chains that can form a covalent bond with a cysteine, the human IgG1 Fc also contained replacement of the cysteine residues to a serine residue at position 220 (C220S) by Eu Index numbering system for immunoglobulin proteins (corresponding to position 5 (C5S) with reference to the wild-type or unmodified Fc set forth in SEQ ID NO: 586). The Fc region also lacked the C-terminal lysine at position 447 (designated K447del) normally encoded in the wild type human IgG1 constant region gene (corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 586). The effectorless (inert) IgG1 Fc in the fusion constructs is set forth in SEQ ID NO:589:

**[0680]** Output cell pools from selected BCMA ECD FACS sorts were grown to terminal density in SCD-Leu selection medium and plasmid DNA was isolated using a yeast plasmid DNA isolation kit (Zymoresearch, USA). For generation of Fc fusions, the affinity matured BCMA ECD variants were PCR amplified with primers containing 40 bp homologous regions on either end with an AfeI and BamHI digested Fc fusion vector encoding and in-frame with the Fc region to carry out in vitro recombination using Gibson Assembly Master Mix (New England Biolabs). The Gibson Assembly reaction was added to the E. coli strain NEB5alpha (New England Biolabs, USA) for heat shock transformation following the manufacturer's instructions.

**[0681]** Dilutions of transformation reactions were plated onto LB-agar containing 100 μg/mL carbenicillin (Teknova, USA) to isolate single colonies for selection. Generally, up to 96 colonies from each transformation were then grown in 96 well plates to saturation overnight at 37°C in LB-broth containing 100 μg/mL carbenicillin (Teknova cat # L8112) and a small aliquot from each well was submitted for DNA sequencing to identify mutation(s) in all clones.

**[0682]** After sequence analysis and identification of clones of interest, plasmid DNA was prepared using the MidiPlus kit (Qiagen).

**[0683]** Recombinant variant Fc fusion proteins were produced from suspension-adapted human embryonic kidney (HEK) 293 cells using the Expi293 expression system (Invitrogen, USA). Supernatant was harvested and the Fc Protein was captured on Mab SelectSure (GE Healthcare cat. no. 17543801). Protein was eluted from the column using 50mM Acetate pH3.6. The MabSelect Sure eluate was pooled and the pH was adjusted to above pH5.0. This material was then polished on a Preparative SEC column, to generate highly purified monomeric material. This material was buffer exchanged into 10mM Acetate, 9% Sucrose pH 5.0. The protein purity was assessed by analytic SEC. Material is vialed and stored at -80.

**[0684]** Amino acid substitutions in selected BCMA vTDs that were identified by the selection are set forth in Table 1. Selected BCMA vTDs formatted as Fc fusion proteins, were tested for binding and functional activity as described in Example 6.

**Example 6. Assessment of Activity of Fc fusion proteins.**

**[0685]** This Example describes characterization of the activity of BCMA domain-containing molecules, such as soluble wild-type (WT) or variant BCMA vTDs formatted as Fc fusions, using a cell line-based *in vitro* bioassay.

**[0686]** Jurkat cells with a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) luciferase-based reporter were purchased (BPS Bioscience). Jurkat/NK-κB cells were transduced with lentivirus to yield stable, cell surface expression of mouse TACI (Jurkat/ NF-κB/TACI). Cells expressing mouse TACI respond to both human and mouse APRIL or BAFF. Following binding of recombinant human or mouse APRIL or BAFF to TACI, endogenous NK-κB transcription factors in the Jurkat cells bind to the DNA response elements controlling transcription of a firefly luciferase gene. Luciferase production was quantitated through the addition of a luciferin-containing substrate which, when oxidized, generates light that can be measured using a microplate reader. A schematic of the Jurkat/NF-κB/TACI assay is shown in **FIG. 1**.

**[0687]** Recombinant human and mouse APRIL and BAFF ligands were purchased: human APRIL (Tonbo Biosciences); human BAFF (BioLegend); mouse APRIL (ProSci Incorporated); and mouse BAFF (R & D Systems).

**[0688]** To determine bioactivity of BCMA WT or vTD domain-containing molecules, recombinant human or mouse APRIL or BAFF at varying concentrations (ranging 1 – 10 nM) in 30 µL were incubated with fixed or titrated (ranging 40 nM – 66 pM) BCMA domain-containing molecules in 30 µL. Ligands and soluble receptors were incubated for 20 minutes with shaking at room temperature (RT). Fifty µL was transferred to a 96-well, white flat-bottomed plated containing $1.5x10^5$ Jurkat/NF-κB/TACI

cells/well in 50 µL media (RPMI1640 + 5% fetal bovine serum [FBS]). Wells were mixed and plates incubated for 5 hours at 37° Celsius (C) in a humidified 5% $CO_2$ incubation chamber. Plates were removed from the incubator and 100 µL of cell lysis and luciferase substrate solution (Bio-Glo™ Luciferase Assay System, Promega) was added to each well and the plates were incubated on an orbital shaker for 10 minutes. Relative luminescence values (RLU) were determined for each test sample by measuring luminescence with a 1 second per well integration time using a Cytation 3 (BioTek Instruments) imaging reader. Decreased RLU in the presence of BCMA WT or vTDs relative to control proteins represent blockade and inhibition of ligand signaling via the transduced TACI receptor in the Jurkat/NF-κB/TACI cells.

**[0689]** As shown in **FIG. 2**, exemplary BCMA-Fc vTDs, inhibit ligand signaling at levels equal to or greater than Fc fusion proteins containing WT BCMA domains.

## Example 7. Generation of Multi-Domain T and B Cell Inhibitory Immunomodulatory Proteins

**[0690]** Multi-domain immunomodulatory proteins were generated containing (1) at least one T cell inhibitory molecule (TIM) that binds to a T cell stimulatory receptor, such as CD28, or a ligand thereof; and (2) at least one B cell inhibitory molecule (BIM) that binds to a B cell stimulatory receptor, such as BCMA or APRIL, or a ligand thereof. Exemplary TIMs included a wild-type (WT) or variant CTLA-4 ECD containing an IgSF domain (CTLA-4 IgD or vIgD, respectively) that binds to CD28, e.g. such as any as described in Example 1. Exemplary BIMs included (i) a wild-type (WT) or variant TACI ECD containing a TD domain (TACI TD or vTD, respectively) that binds to ligands APRIL or BAFF, such as described in Example 5; or (ii) a WT or variant BCMA ECD containing a TD domain (BCMA TD or vTD, respectively) that binds to ligands APRIL or BAFF , such as described in Example 6.

**[0691]** The immunomodulatory proteins were generated as either multimeric molecules via fusion with an Fc protein or as monomeric molecules.

### A. Multimeric Configurations

**[0692]** Various multi-domain immunomodulatory proteins were generated as multimeric molecules via fusion with an Fc protein in various configurations as summarized below. The TIM or BIM of the multi-domain immunomodulatory protein were variously linked to the N- or C-terminus of an Fc region via a peptide linker, such as a GSGGGGS (SEQ ID NO: 590),GGGGSGGGGS (2xGGGGS; SEQ ID NO: 594), $(GGGGS)_4$ (SEQ ID NO:600), or $(GGGGS)_5$ (SEQ ID NO:671).

**[0693]** To generate homodimeric Fc fusions, an exemplary IgG1 Fc region used in generated constructs had the sequence set forth in SEQ ID NO:589 and contained the mutation C220S by EU

numbering, the mutations L234A, L235E, and G237A, by EU numbering, to reduce effector function (the mutations corresponded to C5S, L19A, L20E, G22A, with reference to wild-type human IgG1 Fc set forth in SEQ ID NO:586). and removal of the C-terminal lysine, K447del by EU numbering (corresponding to deletion of position 232, with reference to wild-type or unmodified Fc set forth in SEQ ID NO: 586).

**[0694]** Table E7-A below sets forth exemplary generated multi-domain homodimeric immunomodulatory Fc fusion proteins.

**Table E7-A: Multi-Domain Immunomodulatory Proteins (Homodimer)**

| Description | DNA SEQ ID NO | Protein SEQ ID NO | | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | BCMA (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
|---|---|---|---|---|---|---|---|---|
| | | - His tag | + His tag | | | | | |
| **CTLA-4 ECD and BCMA** | | | | | | | | |
| CTLA-4 186–GSG4S – Fc-(G4S)4 – BCMA 406 | | 601 | | G29W/L98Q/Y105L ECD (186) | | H19L (2-54) (406) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406 | | 602 | | CTLA-4 ECD (1) | | H19L (2-54) (406) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| CTLA-4 186GSG4S – Fc- (G4S)4 – BCMA 381 | | 603 | | G29W/L98Q/Y105L ECD (186) | | S16A, H19Y, R39Q (2-54) (381) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| CTLA-4 186GSG4S – Fc- (G4S)4 – BCMA 411 | | 604 | | G29W/L98Q/Y105L ECD (186) | | H19R (2-54) (411) | GSG4S (590) | Fc (589) |

| Table E7-A: Multi-Domain Immunomodulatory Proteins (Homodimer) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Description | DNA SEQ ID NO | Protein SEQ ID NO | | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | BCMA (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
| | | - His tag | + His tag | | | | | |
| | | | | | | | (G4S)4 (600) | |
| CTLA-4 186GSG4S – Fc- (G4S)4 – BCMA 405 | | 605 | | G29W/L98Q/Y105L ECD (186) | | H19K (2-54) (405) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 381 | | 606 | | CTLA-4 ECD (1) | | S16A, H19Y, R39Q (2-54) (381) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 411 | | 607 | | CTLA-4 ECD (1) | | H19R (2-54) (411) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 405 | | 608 | | CTLA-4 ECD (1) | | H19K (2-54) (405) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| BCMA 381 GSG4S – Fc-(G4S)4 –CTLA-4 186 | | 609 | | G29W/L98Q/Y105L ECD (186) | | S16A, H19Y, R39Q (2-54) (381) | GSG4S (590) (G4S)4 (600) | Fc (589) |

| Table E7-A: Multi-Domain Immunomodulatory Proteins (Homodimer) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Description | DNA SEQ ID NO | Protein SEQ ID NO | | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | BCMA (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
| | | - His tag | + His tag | | | | | |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)2 BCMA 356 | | 631 | | CTLA-4 ECD (1) | | 2-54 WT (356) | GSG4S (590) (G4S)2 (594) | Fc (589) |
| CTLA-4 92 GSG4S Fc (G4S)2 BCMA 356 | | 632 | | G29W/N58S/L63P/Q82R/L98Q/Y105L ECD (92) | | 2-54 WT (356) | GSG4S (590) (G4S)2 (594) | Fc (589) |
| CTLA-4 165 GSG4S Fc (G4S)2 BCMA 356 | | 633 | | T53S/L63P/L98Q ECD (165) | | 2-54 WT (356) | GSG4S (590) (G4S)2 (594) | Fc (589) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)2 BCMA 357 | | 634 | | CTLA-4 ECD (1) | | H19Y (2-54) (357) | GSG4S (590) (G4S)2 (594) | Fc (589) |
| CTLA-4 92 GSG4S Fc (G4S)2 BCMA 357 | | 635 | | G29W/N58S/L63P/Q82R/L98Q/Y105L ECD | | H19Y (2-54) (357) | GSG4S (590) (G4S)2 (594) | Fc (589) |

| Table E7-A: Multi-Domain Immunomodulatory Proteins (Homodimer) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Description | DNA SEQ ID NO | Protein SEQ ID NO | | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | BCMA (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
| | | - His tag | + His tag | | | | | |
| | | | | (92) | | | | |
| CTLA-4 165 GSG4S Fc (G4S)2 BCMA 357 | | 636 | | T53S/L63P/L98Q ECD (165) | | H19Y (2-54) (357) | GSG4S (590) (G4S)2 (594) | Fc (589) |
| BCMA 411 GSG4S Fc (G4S)4 CTLA-4 186 | | 645 | | G29W/L98Q/Y105L ECD (186) | | H19R (2-54) (411) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| BCMA 405 GSG4S Fc (G4S)4 CTLA-4 186 | | 646 | | G29W/L98Q/Y105L ECD (186) | | H19K (2-54) (405) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| BCMA 406 GSG4S Fc (G4S)4 CTLA-4 186 | | 647 | | G29W/L98Q/Y105L ECD (186) | | H19L (2-54) (406) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| BCMA 381 GSG4S Fc (G4S)4 CTLA-4 WT ECD 1 | | 649 | | CTLA-4 ECD (1) | | S16A, H19Y, R39Q (2-54) (381) | GSG4S (590) (G4S)4 (600) | Fc (589) |

| Table E7-A: Multi-Domain Immunomodulatory Proteins (Homodimer) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Description | DNA SEQ ID NO | Protein SEQ ID NO | | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | BCMA (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
| | | - His tag | + His tag | | | | | |
| BCMA 411 GSG4S Fc (G4S)4 CTLA-4 WT ECD 1 | | 650 | | CTLA-4 ECD (1) | | H19R (2-54) (411) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| BCMA 405 GSG4S Fc (G4S)4 CTLA-4 WT ECD 1 | | 651 | | CTLA-4 ECD (1) | | H19K (2-54) (405) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| BCMA 406 GSG4S Fc (G4S)4 CTLA-4 WT ECD 1 | | 652 | | CTLA-4 ECD (1) | | H19L (2-54) (406) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| BCMA 357 (G4S)3 CTLA-4 92 GSG4S Fc | | 655 | | G29W/N58S/L63P/Q82R/L98Q/Y105L ECD (92) | | H19Y (2-54) (357) | (G4S)3 (595) GSG4S (590) | Fc (589) |
| BCMA 357 GSG4S Fc (G4S)3 CTLA-4 92 | | 656 | | G29W/N58S/L63P/Q82R/L98Q/Y105L ECD | | H19Y (2-54) (357) | (G4S)3 (595) GSG4S (590) | Fc (589) |

| Table E7-A: Multi-Domain Immunomodulatory Proteins (Homodimer) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Description | DNA SEQ ID NO | Protein SEQ ID NO | | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | BCMA (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
| | | - His tag | + His tag | | | | | |
| | | | | (92) | | | | |
| CTLA-4 92 (G4S)4 BCMA 357 GSG4S Fc | | 657 | | G29W/N58S/L63P/Q82R/L98Q/Y105L ECD (92) | | H19Y (2-54) (357) | (G4S)4 (600) GSG4S (590) | Fc (589) |
| CTLA-4 92 GSG4S Fc (G4S)3 BCMA 357 | | 658 | | G29W/N58S/L63P/Q82R/L98Q/Y105L ECD (92) | | H19Y (2-54) (357) | GSG4S (590) (G4S)3 (595) | Fc (589) |
| CTLA-4 92 GSG4S Fc (G4S)5 BCMA 357 | | 659 | | G29W/N58S/L63P/Q82R/L98Q/Y105L ECD (92) | | H19Y (2-54) (357) | GSG4S (590) (G4S)5 (671) | Fc (589) |
| **CTLA-4 ECD and TACI** | | | | | | | | |
| CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | | 610 | | G29W/L98Q/Y105L ECD (186) | K77E, F78Y, Y102D (68-110) | | GSG4S (590) (G4S)4 | Fc (589) |

| Table E7-A: Multi-Domain Immunomodulatory Proteins (Homodimer) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Description | DNA SEQ ID NO | Protein SEQ ID NO | | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | BCMA (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
| | | - His tag | + His tag | | | | | |
| | | | | | (541) | | (600) | |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | | 611 | | CTLA-4 ECD (1) | K77E, F78Y, Y102D(68-110) (541) | | GSG4S (590) (G4S)4 (600) | Fc (589) |
| CTLA-4 186 GSG4S Fc (G4S)4 (TACI 542) | | 612 | | G29W/L98Q/Y105L ECD (186) | Q75E, R84Q(68-110) (542) | | GSG4S (590) (G4S)4 (600) | Fc (589) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 (TACI 542) | | 613 | | CTLA-4 ECD (1) | Q75E, R84Q(68-110) (542) | | GSG4S (590) (G4S)4 (600) | Fc (589) |
| TACI 542 GSG4S Fc (G4S)4 (CTLA-4 186) | | 614 | | G29W/L98Q/Y105L ECD (186) | Q75E, R84Q(68-110) (542) | | GSG4S (590) (G4S)4 (600) | Fc (589) |
| TACI 516 (G4S)2 Fc (G4S)2 CTLA 4 668 | | 615 | | C122S ECD (668) | 29-110 WT (516) | | (G4S)2 (594) | Fc (589) |
| TACI 528 (G4S)2 Fc | | 616 | | CTLA-4 ECD | 68-110 WT | | (G4S)2 (594) | Fc (589) |

| Table E7-A: Multi-Domain Immunomodulatory Proteins (Homodimer) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Description | DNA SEQ ID NO | Protein SEQ ID NO | | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | BCMA (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
| | | - His tag | + His tag | | | | | |
| (G4S)2 CTLA 4 WT ECD 1 | | | | (1) | (528) | | | |
| TACI 528 (G4S)2 Fc (G4S)2 CTLA 4 668 | | 617 | | C122S ECD (668) | 68-110 WT (528) | | (G4S)2 (594) | Fc (589) |
| CTLA-4 WT ECD 1 (G4S)2 Fc (G4S)2 TACI 516 | | 624 | | CTLA-4 ECD (1) | 29-110 WT (516) | | (G4S)2 (594) | Fc (589) |
| CTLA 4 668 (G4S)2 Fc (G4S)2 TACI 516 | | 625 | | C122S ECD (668) | 29-110 WT (516) | | (G4S)2 (594) | Fc (589) |
| CTLA-4 WT ECD 1 (G4S)2 Fc (G4S)2 TACI 528 | | 626 | | CTLA-4 ECD (1) | 68-110 WT (528) | | (G4S)2 (594) | Fc (589) |
| CTLA 4 668 (G4S)2 Fc (G4S)2 TACI 528 | | 627 | | C122S ECD (668) | 68-110 WT (528) | | (G4S)2 (594) | Fc (589) |

| Table E7-A: Multi-Domain Immunomodulatory Proteins (Homodimer) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Description | DNA SEQ ID NO | Protein SEQ ID NO | | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | BCMA (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
| | | - His tag | + His tag | | | | | |
| CTLA 4 92 GSG4S Fc (G4S)2 TACI 516 | | 637 | | G29W/N58S/L63P/Q82R/L98Q/Y105L ECD (92) | 29-110 WT (516) | | GSG4S (590) (G4S)2 (594) | Fc (589) |
| CTLA 4 165 GSG4S Fc (G4S)2 TACI 516 | | 638 | | T53S/L63P/L98Q ECD (165) | 29-110 WT (516) | | GSG4S (590) (G4S)2 (594) | Fc (589) |
| CTLA-4 113 GSG4S Fc (G4S)4 TACI 535 (68-110) | | 643 | | L12F/R16H/G29W/M56T/L98Q/Y105L ECD (113) | R84G (68-110) (535) | | GSG4S (590) (G4S)2 (594) | Fc (589) |
| CTLA-4 113 GSG4S Fc (G4S)4 TACI 688 (29-110) | | 644 | | L12F/R16H/G29W/M56T/L98Q/Y105L ECD (113) | R84G (29-110) (688) | | GSG4S (590) (G4S)2 (594) | Fc (589) |
| TACI 541 GSG4S Fc (G4S)4 CTLA-4 186 | | 648 | | G29W/L98Q/Y105L ECD (186) | K77E, F78Y, Y102D(68-110) (541) | | GSG4S (590) (G4S)2 (594) | Fc (589) |

| Table E7-A: Multi-Domain Immunomodulatory Proteins (Homodimer) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Description | DNA SEQ ID NO | Protein SEQ ID NO | | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | BCMA (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
| | | - His tag | + His tag | | | | | |
| TACI 541 GSG4S Fc (G4S)4 CTLA-4 WT ECD 1 | | 653 | | CTLA-4 ECD (1) | K77E, F78Y, Y102D(68-110) (541) | | GSG4S (590) (G4S)2 (594) | Fc (589) |
| TACI 542 GSG4S Fc (G4S)4 CTLA-4 WT ECD 1 | | 654 | | CTLA-4 ECD (1) | Q75E, R84Q(68-110) (542) | | GSG4S (590) (G4S)2 (594) | Fc (589) |

**[0695]** For generation of heterodimeric multi-domain Fc fusion proteins, multimeric multi-domain immunomodulatory proteins were generated as heterodimeric molecules by "knobs-into-hole" engineering. In such an example, the heterodimer was generated by co-expressing a TIM and a BIM that each were fused to either (1) a first "knob" Fc subunit (set forth in SEQ ID NO:669 containing the mutations S354C and T366W by EU numbering, corresponding to S139C and T151W with reference to wild-type human IgG1 Fc set forth in SEQ ID NO:586); and (2) a second "hole" Fc subunit (set forth in SEQ ID NO:670, containing the mutations Y349C, T366S, L368A and Y407V by EU numbering, corresponding to Y134C, T151S, L153A and Y192V with reference to wild-type human IgG1 Fc set forth in SEQ ID NO:586) for expression of a heterodimeric molecule. In addition, both the knob and hole Fc also contained mutations L19A, L20E, G22A to reduce effector function and contained replacement of the cysteine residue to a serine residue at position 5 (C5S), each compared to the wild-type or unmodified Fc set forth in SEQ ID NO: 586 (corresponding to C220S, L234A, L235E and G237A by EU numbering, respectively).

Knob Fc (SEQ ID NO:669):

EPKSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Hole Fc (SEQ ID NO:670):
EPKSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

**[0696]** Table E7-B sets forth exemplary generated multi-domain heterodimeric immunomodulatory fusion proteins.

**Table E7-B: Heterodimeric Multi-Domain Immunomodulatory Proteins CTLA/BCMA/TACI**

| | | | | TIM | BIM | | | |
|---|---|---|---|---|---|---|---|---|
| **Description** | **DNA SEQ ID NO** | **Protein SEQ ID NO Protein SEQ ID NO +His tag** | | **CTLA-4 (SEQ ID NO)** | **TACI (SEQ ID NO)** | **BCMA (SEQ ID NO)** | **Linker (SEQ ID NO)** | **Fc (SEQ ID NO)** |
| CTLA-4 92 Fc + BCMA 357 Fc | CTLA-4 92 GSG4S Fc K | 662 | | G29W/N58S/L63P/Q82R/L98Q/Y105L ECD (92) | | | GSG4S (590) | Fc K (669) |
| | BCMA 357 GSG4S Fc H | 660 | | | | H19Y (2-54) (357) | GSG4S (590) | Fc H (670) |
| CTLA-4 113 Fc + BCMA 357 Fc | CTLA-4 113 | 663 | | L12F/R16H/G29W/M56T/L9 | | | GSG4S (590) | Fc K (669) |

| Table E7-B: Heterodimeric Multi-Domain Immunomodulatory Proteins CTLA/BCMA/TACI | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | TIM | BIM | | | |
| **Description** | **DNA SEQ ID NO** | **Protein SEQ ID NO Protein SEQ ID NO +His tag** | | **CTLA-4 (SEQ ID NO)** | **TACI (SEQ ID NO)** | **BCMA (SEQ ID NO)** | **Linker (SEQ ID NO)** | **Fc (SEQ ID NO)** |
| | GSG4S Fc K | | | 8Q/Y105L ECD (113) | | | | |
| | BCMA 357 GSG4S Fc H | 660 | | | | H19Y (2-54) (357) | GSG4S (590) | Fc H (670) |
| | | | | | | | | |

**[0697]** CTLA-4-Fc (Abatacept) or BCMA-Fc (Table E8-A) were used as controls of the multi-domain immunomodulatory Fc fusion proteins.

**[0698]** Expression constructs encoding Fc fusion proteins of interest were transiently expressed in Expi293 HEK293 cells (e.g. Invitrogen) with Expifectamine™ reagents and media following the manufacturer's instructions. Supernatants were harvested and protein was captured and eluted from a Protein A column using an AKTA protein purification system. The eluted material was then separated by an additional preparative SEC step to generate non-aggregated (monomeric), highly purified material. This material was buffer exchanged into 10mM Acetate, 9% Sucrose, pH 5.0. (A5Su) The protein was vialed in a sterile biosafety cabinet and frozen at -80 C. A vial was thawed and assessed by analytical SEC to demonstrate the material was stable and predominantly non-aggregated (monomeric) after thaw.

| Table E8-A: BCMA Fc | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Description | DNA SEQ ID NO | Protein SEQ ID NO | Protein SEQ ID NO | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | BCMA (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | +His tag | | | | | |
| Fc (G4S)2 BCMA (356) | | 628 | | | | 2-54 WT (356) | (G4S)2 (594) | Fc (589) |
| Fc (G4S)2 BCMA (357) | | 629 | | | | H19Y (2-54) (357) | (G4S)2 (594) | Fc (589) |

B. Monomeric Configurations

**[0699]** In some configurations, the generated multi-domain immunomodulatory proteins were generated as monomeric molecules containing a TIM and a BIM linked together with a peptide linker. In this example, the constructs were generated with the linker (EAAAK)6 (SEQ ID NO:665). In some cases, the monomeric immunomodulatory proteins also contained an N- or C-terminal moiety for detection and/or purification, such as a poly-histidine tag (HHHHHH; SEQ ID NO:702) and/or a flag-tag (DYKDDDDK; SEQ ID NO: 588).

**[0700]** Table E9 describes exemplary generated multi-domain immunomodulatory monomeric proteins.

**Table E9: Monomeric Multi-Domain Immunomodulatory Proteins**

| **Description** | **DNA SEQ ID NO** | **Protein SEQ ID NO** | | **CTLA-4 (SEQ ID NO)** | **TACI (SEQ ID NO) BCMA (SEQ ID NO)** | **Linker (SEQ ID NO)** |
|---|---|---|---|---|---|---|
| | | - His tag | + Flag His tag | | | |
| TACI 516 (EAAAK)6 CTLA 4 WT ECD 1 | | 703 | 618 | CTLA-4 ECD (1) | 29-110 WT (516) | (EAAAK)6 (665) |
| TACI 516 (EAAAK)6 CTLA 4 668 | | 704 | 619 | C122S ECD (668) | 29-110 WT (516) | (EAAAK)6 (665) |

| | | | | | | |
|---|---|---|---|---|---|---|
| TACI 528 (EAAAK)6 CTLA 4 WT ECD 1 | | 705 | 620 | CTLA-4 ECD (1) | 68-110 WT (528) | (EAAAK) 6 (665) |
| TACI 528 (EAAAK)6 CTLA 4 668 | | 706 | 621 | C122S ECD (668) | 68-110 WT (528) | (EAAAK) 6 (665) |
| TACI 516 (G4S)3 CTLA 4 668 | | 707 | 622 | C122S ECD (668) | 29-110 WT (516) | (G4S)3 (595) |
| TACI 528 (G4S)3 CTLA 4 668 | | 708 | 623 | C122S ECD (668) | 68-110 WT (528) | (G4S)3 (595) |

**Example 8. Bioactivity Assessment of BCMA/TACI Blockade of TACI-mediated stimulation by BCMA- or TACI-containing molecules stacked with CTLA-4 Domains.**

**[0701]** The cell-line based bioassay described in Example 6 was used to assess the functional characterization of TACI- or BCMA-containing WT, vTD, or multi-domain proteins "stacked" with a CTLA-4 WT or vIgSF domain for blockade of APRIL or BAFF-mediated ligand signaling via the TACI receptor in the Jurkat/NF-κB/TACI cells. Exemplary stack molecules described in Example 7 were assessed. APRIL or BAFF-mediated ligand signaling was quantitated by monitoring luciferase production in the cells.

**A. Bioactivity of Exemplary Multi-Domain Molecules**

**[0702]** In one experiment, exemplary molecules set forth in Table E8 were assessed using the Jurkat /NF-κB/TACI reporter cells for blockade of APRIL- or BAFF-mediated signaling. **Table E10-A** provides the values for half maximal inhibitory concentration (IC50) for inhibition of APRIL- and BAFF- mediated TACI signaling. Also shown is a comparison to the corresponding WT BCMA-Fc or WT TACI-Fc controls (Δ Parental vTD) for each experiment. In some instances, the proteins tested were not compared to their parental of WT controls and appear as (-) in the Table below.

| Table E10-A. Bioactivity of Multi-Domain Immunomodulatory Proteins | | | | | |
|---|---|---|---|---|---|
| Description | SEQ ID NO | IC50 (nM) APRIL | IC50 (nM) APRIL ( Δ Parental vTD) | IC50 (nM) BAFF | IC50 (nM) BAFF ( Δ Parental vTD) |
| TACI 516 (EAAAK)6 CTLA 4 WT ECD 1 | 703/618 | 46.15 | 46.2 (5.0) | 7.781 | 7.8 (3.0) |
| TACI 516 (EAAAK)6 CTLA 4 668 | 704/619 | 138 | 138.0 (15.0) | 3.422 | 3.4 (1.3) |
| TACI 528 (EAAAK)6 CTLA 4 WT ECD 1 | 705/620 | ND | ND | 5.305 | 5.3 (2.1) |
| TACI 528 (EAAAK)6 CTLA 4 668 | 706/621 | ND | ND | 5.176 | 5.2 (2.0) |
| TACI 516 (G4S)3 CTLA 4 668 | 707/622 | ND | ND | 8.747 | 8.7 (3.4) |
| TACI 528 (G4S)3 CTLA 4 668 | 708/623 | ND | ND | 8.412 | 8.4 (3.3) |
| CTLA-4 WT ECD 1 (G4S)2 Fc (G4S)2 TACI 516 | 624 | 19.15 | 19.2 (2.1) | 9.459 | 9.5 (3.7) |
| CTLA 4 668 (G4S)2 Fc (G4S)2 TACI 516 | 625 | 19.97 | 20.0 (2.2) | 10.05 | 10.1 (3.9) |
| CTLA-4 WT ECD 1 (G4S)2 Fc (G4S)2 TACI 528 | 626 | 37.53 | 37.5 (4.1) | 2.06 | 2.1 (0.8) |
| CTLA 4 668 (G4S)2 Fc (G4S)2 TACI 528 | 627 | 8.655 | 8.7 (0.9) | 2.237 | 2.2 (0.9) |
| TACI WT Fc | 516 | 9.173 | 9.2 (1.0) | 2.579 | 2.6 (1.0) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)2 BCMA 356 | 631 | 0.8383 | 0.8 (0.0) | 33.63 | 33.6 (0.8) |
| CTLA-4 165 GSG4S Fc (G4S)2 BCMA 356 | 633 | 0.8939 | 0.9 (0.0) | 36.83 | 36.8 (0.9) |
| CTLA-4 92 GSG4S Fc (G4S)2 BCMA 356 | 632 | 0.8094 | 0.8 (0.0) | 33.46 | 33.5 (0.8) |
| Fc (G4S)2 BCMA (356) | 628 | 42.71 | 42.7 (1.0) | 42.71 | 42.7 (1.0) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)2 BCMA 357 | 634 | 0.6879 | 0.7 (1.0) | 3.642 | 3.6 (0.7) |

| Table E10-A. Bioactivity of Multi-Domain Immunomodulatory Proteins | | | | | |
|---|---|---|---|---|---|
| Description | SEQ ID NO | IC50 (nM) APRIL | IC50 (nM) APRIL ( Δ Parental vTD) | IC50 (nM) BAFF | IC50 (nM) BAFF ( Δ Parental vTD) |
| CTLA-4 92 GSG4S Fc (G4S)2 BCMA 357 | 635 | 0.6831 | 0.7 (1.0) | 6.274 | 6.3 (1.2) |
| CTLA-4 165 GSG4S Fc (G4S)2 BCMA 357 | 636 | 0.6057 | 0.6 (0.9) | 11.84 | 11.8 (2.2) |
| Fc (G4S)2 BCMA (357) | 629 | 0.6957 | 0.7 (1.0) | 5.439 | 5.4 (1.0) |
| CTLA 4 165 GSG4S Fc (G4S)2 TACI 516 | 638 | 4.644 | 4.6 (0.9) | 0.9277 | 0.9 (0.5) |
| CTLA 4 92 GSG4S Fc (G4S)2 TACI 516 | 637 | 6.752 | 6.8 (1.3) | 1.732 | 1.7 (1.0) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)2 BCMA 356 | 631 | 5.106 | 5.1 (1.0) | 1.698 | 1.7 (1.0) |
| CTLA-4 113 GSG4S Fc (G4S)4 TACI 535 (68-110) | 643 | 8.632 | 8.6 (NA) | 0.5059 | 0.51 (0.4) |
| CTLA-4 113 GSG4S Fc (G4S)4 TACI 535 (29-110) | 644 | 8.253 | 8.5 (1.4) | 0.566 | 0.57 (0.4) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)2 BCMA 356 | 631 | 6.087 | 6.1 (1.0) | 1.389 | 1.39 (1.0) |
| CTLA-4 186GSG4S – Fc-(G4S)4 – BCMA 381 | 603 | 1.27 | 1.3 (-) | 0.2818 | 0.28 (-) |
| CTLA-4 186GSG4S – Fc-(G4S)4 – BCMA 411 | 604 | 2.371 | 2.4 (-) | 0.1329 | 0.13 (-) |
| CTLA-4 186GSG4S – Fc-(G4S)4 – BCMA 405 | 605 | 18.39 | 18.4 (-) | 0.1604 | 0.16 (-) |
| CTLA-4 186– GSG4S – Fc-(G4S)4 – BCMA 406 | 601 | 0.9969 | 1.0 (-) | 0.4901 | 0.49 (-) |
| CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 610 | 1.558 | 1.6 (-) | 0.1027 | 0.10 (-) |
| CTLA-4 186 GSG4S Fc (G4S)4 (TACI 542 | 612 | 2.49 | 2.5 (-) | 0.1532 | 0.15 (-) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 381 | 606 | 0.8491 | 0.8 (-) | 0.234 | 0.23 (-) |

| Table E10-A. Bioactivity of Multi-Domain Immunomodulatory Proteins | | | | | |
|---|---|---|---|---|---|
| Description | SEQ ID NO | IC50 (nM) APRIL | IC50 (nM) APRIL ( Δ Parental vTD) | IC50 (nM) BAFF | IC50 (nM) BAFF ( Δ Parental vTD) |
| | | | | | |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 411 | 607 | 2.005 | 2.0 (-) | 0.1746 | 0.17 (-) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 405 | 608 | 23.13 | 23.1 (-) | 0.1956 | 0.20 (-) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406 | 602 | 1.185 | 1.2 (-) | 0.678 | 0.68 (-) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | 611 | 1.646 | 1.6 (-) | 0.1285 | 0.13 (-) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 (TACI 542 | 613 | 2.732 | 2.7 (-) | 0.1599 | 0.16 (-) |
| BCMA 381 GSG4S – Fc-(G4S)4 –CTLA-4 186 | 609 | 0.8762 | 0.9 (-) | 0.06175 | 0.06 (-) |
| TACI 542 GSG4S Fc (G4S)4 (CTLA-4 186 | 614 | 1.2 | 1.2 (-) | 0.1786 | 0.18 (-) |
| BCMA 357 (G4S)3 CTLA-4 92 GSG4S Fc | 655 | 0.6888 | 0.69 (0.7) | 0.3963 | 0.4 (0.4) |
| BCMA 357 GSG4S Fc (G4S)3 CTLA-4 92 | 656 | 0.5824 | 0.58 (0.6) | 0.4155 | 0.4 (0.4) |
| CTLA-4 92 (G4S)4 BCMA 357 GSG4S Fc | 657 | 0.63 | 0.63 (0.6) | 3.754 | 3.8 (3.8) |
| CTLA-4 92 GSG4S Fc (G4S)3 BCMA 357 | 658 | 0.5499 | 0.55 (0.5) | 0.5481 | 0.6 (0.6) |
| CTLA-4 92 GSG4S Fc (G4S)5 BCMA 357 | 659 | 0.6873 | 0.69 (0.7) | 0.7649 | 0.8 (0.8) |
| BCMA WT-Fc | 356 | 1.026 | 1.03 (1.0) | 0.9946 | 1.0 (1.0) |
| CTLA-4 92 Fc K + BCMA 357 Fc H | 662<br>660 | 4.19 | 4.2 (4.2) | 5.385 | 5.4 (5.4) |
| CTLA-4 113 Fc K/ BCMA 357 Fc H | 663 | 5.203 | 5.2 (5.2) | 5.153 | 5.2 (5.2) |

| Table E10-A. Bioactivity of Multi-Domain Immunomodulatory Proteins | | | | | |
|---|---|---|---|---|---|
| Description | SEQ ID NO | IC50 (nM) APRIL | IC50 (nM) APRIL ( Δ Parental vTD) | IC50 (nM) BAFF | IC50 (nM) BAFF ( Δ Parental vTD) |
| | 660 | | | | |
| BCMA WT-Fc | 356 | 1.026 | 1.0 (1.0) | 0.9946 | 1.0 (1.0) |

**B. Activity of Variant BCMA to Inhibit BAFF and APRIL, or BCMA or TACI vTDs Combined as a Multi-Domain Immunomodulatory Protein with Variant CTLA-4 vIgD**

**[0703]** As shown in **FIG. 3A**, a BCMA vTD demonstrated comparable activity whether it is used as a single domain or included as part of a multidomain stacked molecule. As shown in **FIG. 3B,** BCMA vTDs included in stack molecules are interchangeable and can be used to modulate ligand inhibition.

**[0704]** As shown in **FIG. 3C**, TACI vTDs included as mutlidomain stacked molecules demonstrate the ability to block both APRIL and BAFF. . As shown in **FIG. 3D**, TACI vTDs included in stack molecules are interchangeable and can be used to modulate ligand inhibition.

**[0705]** As shown in **FIG. 4**, exemplary BCMA vTD-Fcs and stacks containing BCMA or TACI vTDs with CTLA-4 vIgD inhibit mouse APRIL and BAFF ligand signaling. Together, the results show the ability of the multidomain stack molecules to block APRIL and BAFF ligand signaling (in this example exemplified via TACI-mediated signaling) similarly to the single domain controls.

C. **Comparison of Variant BCMA vTDs and BCMA and TACI vTD Multi-Domain Immunomodulatory Proteins for Blockade of APRIL and BAFF Mediated Signaling Relative to Fc fusion containing ECD portions present in Atacicept or Telitacicept**

**[0706]** In another similar study, exemplary generated molecules as described in Example 7 were assessed for their ability to block APRIL or BAFF-mediated ligand signaling in Jurkat/NF-κB/TACI cells. For comparison, control molecules were generated containing wild-type TACI ECD fused the Fc sequence set forth in SEQ ID NO: 589. In one control, the fusion protein contained WT TACI (TACI 30-110, SEQ ID NO:718; corresponding to the TACI ECD portion in atacicept, SEQ ID NO:720). In another control, the fusion protein contained WT TACI (TACI 13-118, SEQ ID NO:719), corresponding to the TACI ECD portion in telitacicept). Activity was compared to the control molecules. Activity also was compared to the anti-BAFF monoclonal antibody belimumab.

**[0707]** Exemplary BCMA molecules, either WT or variant BCMA vTDs alone or multi-domain molecules further combined with a variant CTLA-4 IgSF, were titrated (between 100,000pM – 32pM), added to 2nM recombinant human APRIL or BAFF and assayed as described above for the Jurkat/NF-κB

assay. As shown in **FIG. 5A**, the exemplary molecules containing a BCMA TD alone or as a multdomain protein exhibited enhanced APRIL blockade, but not BAFF blockade, compared to TACI 30-110-Fc, TACI 13-118-Fc and belimumab.

**[0708]** Exemplary TACI molecules, either WT vTDs or WT and vTD multi-domain molecules further combined with a variant CTLA-4 IgSF, were titrated (between 100,000pM – 32pM), added to 2nM recombinant human APRIL or BAFF and assayed as described above for the Jurkat/NF-κB assay. As shown in **FIG. 5B**, the exemplary molecules containing TACI vTDs as a multi-domain protein exhibited enhanced APRIL and BAFF blockade greater than TACI 30-100-Fc, TACI 13-118-Fc and belimumab. WT TACI-Fc containing only the CRD2 domain of TACI also exhibited enhanced APRIL blockade greater than TACI 30-100-Fc and TACI 13-118-Fc. These results are consistent with a finding that the minimal CRD2 domain (containing amino acids residues 68-110) exhibits improved blockade of APRIL compared to TACI ECD molecules also containing portions of the CRD1 domain as present in atacicept and telitacicept.

**[0709]** **Table E10-B** provides the values for half maximal inhibitory concentration (IC50) for inhibition of APRIL- and BAFF- mediated TACI signaling for the exemplary molecules described in **FIG. 5A** and **FIG. 5B**. Also shown in parentheses is the relative blockage compared to atacicept (Δ atacicept) for each tested molecule.

**Table E10-B. Bioactivity of Multi-Domain Immunomodulatory Proteins vs atacicept**

| Description | SEQ ID NO | IC50 (nM) APRIL | IC50 (nM) APRIL (Δ TACI 30-110-Fc) | IC50 (nM) BAFF (Δ TACI 30-110-Fc) |
|---|---|---|---|---|
| 381 BCMA-Fc | 381 | 180 | 180(0.05) | 1778(0.31) |
| 406 BCMA-Fc | 406 | 190 | 190(0.05) | 1532(0.27) |
| 411 BCMA-Fc | 411 | 209 | 209(0.05) | 781(0.14) |
| CTLA-4 186– GSG4S – Fc- (G4S)4 – BCMA 406 | 601 | 269 | 269(0.07) | 2628(0.46) |
| 356 BCMA-Fc | 356 | 183 | 183(0.05) | 823(0.14) |
| CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 610 | 272 | 272(0.07) | 1259(0.22) |
| 528 TACI CRD2-Fc | 528 | 369 | 369(0.10) | 1328(0.23) |
| TACI 13-118-Fc | 719 | 9103 | 9103(2.37) | 7699(1.33) |
| Belimumab | | 214911 | 214911(55.84) | 2496(0.43) |
| TACI 30-110-Fc | 718 | 3849 | 3849(1.00) | 5771(1.00) |

**Example 9. Bioactivity Assessment of CD80/CD86 Blockade of CD28-mediated Costimulation by CTLA-4 when stacked with Wild-Type or Affinity-Matured BCMA or TACI Domains.**

**[0710]** This Example describes functional characterization of WT, vIgD, or stack proteins containing CTLA-4 by blockade of CD80/CD86 and inhibition of CD28-mediated costimulation with a cell line-based *in vitro* bioassay.

**[0711]** Jurkat cells expressing luciferase driven by an interleukin-2 (IL-2) promoter were purchased (Promega). Jurkat cells, a human T cell leukemia cell line, endogenously express T-cell receptor (TCR) – CD3 complexes and CD28. Upon interaction with CD80 or CD86, CD28 signaling mediates transcriptional upregulation of the IL-2 promoter. Activation of Jurkat cells via anti-CD3 and costimulation via CD80 or CD86 results in luciferase production. Luciferase is quantitated as described in Example 6. K562 cells, a human myelogenous leukemia cell line, were transduced to stably express cell surface anti-human CD3 (OKT3) single chain fragment variable (scFv) and human CD80 or CD86 (K562/OKT3/CD80 or CD86) to generate artificial antigen presenting cells (aAPCs). A schematic of the Jurkat/IL-2 assay is shown in **FIG. 6**.

**[0712]** To determine bioactivity of CTLA-4 WT or vIgDdomains, stack proteins containing these domains were titrated (ranging 100 nM – 256 pM) and plated into a 96-well, white flat-bottom plate in 33 µL of media (RPMI1640 + 5% FBS). aAPCs were added to proteins at $2x10^4$ cell/well in 33 µL media and incubated for 20 minutes at RT, with shaking. Jurkat/IL-2 cells were added at $2x10^5$ cell/well to bring the final volume/well to 100uL. APCs and Jurkat cells were incubated for 5 hours at 37°C in a humidified 5% $CO_2$ incubation chamber. Plates were processed and luminescence quantified as described above. Decreased RLU in the presence of CTLA-4 WT or vIgD, or CTLA-4 stacks relative to control proteins represent blockade of CD80 or CD86 and inhibition of CD28-mediated costimulation in the Jurkat/IL-2 cells.

**[0713]** **Table E11** provides the values for half maximal inhibitory concentration (IC50) for inhibition of CD80- and CD86- mediated CD28 signaling. Also shown is a comparison to the WT-CTLA-4-Fc reference control abatacept (Δ Parental CTLA-4-Fc) for each experiment. In some instances, the proteins tested were not compared to their parental of WT controls and appear as (-) or IC50 valued were not determined (ND).

| Table E11 Bioactivity of Multi-Domain Immunomodulatory Proteins | | | | | |
|---|---|---|---|---|---|
| Description | SEQ ID NO | CD80 IC50 ( nM) | CD80 IC50 (nM) ( Δ Parental CTLA-4) | CD86 IC50 ( Δ WT ) | CD86 IC50 (nM) ( Δ Parental CTLA-4) |
| CTLA-4 186 GSG4S – Fc-(G4S)4 – BCMA 381 | 603 | 26.1 | 26.1 (0.8) | 11.9 | 11.9 (1.5) |
| CTLA-4 186GSG4S – Fc-(G4S)4 – BCMA 411 | 604 | 32.7 | 32.7 (1.0) | 9 | 9.0 (1.1) |
| CTLA-4 186GSG4S – Fc-(G4S)4 – BCMA 405 | 605 | 70.1 | 70.1 (2.1) | 10.8 | 10.8 (1.3) |
| CTLA-4 186– GSG4S – Fc-(G4S)4 – BCMA 406 | 601 | 25.2 | 25.2 (0.8) | 8.1 | 8.1 (1.3) |
| CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 610 | 56.9 | 56.9 (1.7) | 10.2 | 10.2 (1.2) |
| CTLA-4 186 GSG4S Fc (G4S)4 (TACI 542 | 612 | 82.6 | 82.6 (2.5) | 9 | 9.0 (1.1) |
| 186 CTLA-4-Fc | 186 | 33.6 | 33.6 (1.0) | 8.2 | 8.2 (1.0) |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 381 | 606 | 9.4 | 9.4 (1.1) | ND | ND |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 411 | 607 | 10.9 | 10.9 (1.3) | ND | ND |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 405 | 608 | 5.7 | 5.7 (0.7) | ND | ND |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406 | 602 | 11.9 | 11.9 (1.4) | ND | ND |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | 611 | 10.3 | 10.3 (1.2) | ND | ND |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 (TACI 542 | 613 | 9.5 | 9.5 (1.1) | ND | ND |
| Abatacept | | 8.6 | 8.6 (1.0) | ND | ND |
| CTLA-4 92 GSG4S Fc (G4S)2 BCMA 357 | 635 | 22.7 | 22.7 (-) | 16.1 | 16.1 (-) |

**[0714]** **FIGS. 7A** and **7B** provide further results of exemplary molecules. Blockade of CD80 and CD86 and inhibition of CD28-mediated costimulation for exemplary molecules containing a WT CTLA-4 IgD (**FIG. 7A**) or a CTLA-4 vIgD (**FIG. 7B**).

**[0715]** Together, the results show the ability of the multidomain stack molecules to block CD28-mediated costimulation similarly, or in some cases superior to, the single domain CTLA-4 ECD-Fc reference control abatacept. Specifically, stack molecules containing a CTLA-4 vIgD exhibited substantially improved ability to block CD86-mediated CD28 co-stimulation consistent with their increased affinity for CD86 compared to wild-type CTLA-4.

## Example 10. Bioactivity Assessment of BCMA or TACI Domain-Containing Molecules in a Primary Follicular Helper T Cell (Tfh) and B Cell Co-Culture Assay.

**[0716]** Bioactivity of WT BCMA-Fc, WT TACI-Fc, BCMA vTD-Fc, or exemplary multidomain stack molecules containing BCMA or TACI vTD with CTLA-4 IgSF domains were tested in a primary T and B cell assay. Exemplary multidomain molecules described in Example 7 and Table E7 were tested.

**[0717]** Primary cells were obtained from a leukocyte reduction system (LRS) cone (Bloodworks Northwest). The cone was processed and peripheral blood mononuclear cells (PBMCs) were isolated via density gradient centrifugation. Total B cells were isolated from PBMCs using a negative selection kit (StemCell Technologies, catalog #17954) following the manufacturer's supplied protocol. Total CD4+ T cells were isolated from whole PBMCs using a negative selection kit (StemCell Technologies, catalog #17952). CD4+ T cells were further purified to yield Tfh via CXCR5 positive selection. CXCR5 beads were prepared using a "Do-It-Yourself" kit (StemCell Technologies, catalog #17699) by binding purified anti-human CXCR5 antibody (Biolegend) to magnetic particles. Tfh (CXCR5+CD4+ T) cells were isolated following the manufacturer's supplied protocol. Artificial antigen presenting cells (aAPCs) (K562/CD80) were prepared by fixation with mitomycin C (100 µg/mL final) for 30 minutes at 37°C. aAPCs were washed three times with RPMI containing FBS and suspended in serum-free X-Vivo 15™ supplemented medium (X-Vivo 15™ containing 1X GlutaMAX, 1X Penicillin-Streptomycin) after the final wash.

**[0718]** B cells were plated into 96-well tissue culture treated plates at 5.5 – 7.0 $x10^4$ cells/well with recombinant IL-21 (BioLegend, 80ng/ml final) in 50 µL media. Tfh cells were added at 2.0 – 7.0 $x10^3$ cell/well in 50 µL. Fc fusion proteins containing WT BCMA or TACI, BCMA vTDs, or CTLA-4-Fc-BCMA/TACI multidomain stacks were titrated and added in 50 µL (ranging 100 nM – 32 pM) in triplicate. APCs were added at 2.0 – 5.0 $x10^3$ cell/well with soluble anti-CD3 antibody (OKT3) (BioLegend, 10nM final) in 50 µL. Plates were incubated 7 days at 37°C with 5% $CO_2$.

**[0719]** Tfh – B cell co-culture plates were centrifuged and supernatants were transferred to 96-well polypropylene plates and frozen at -20°C until secreted immunoglobulin could be measured by multiplex analysis (MAGPIX® System, Millipore EMD). Cell recovery and activation was determined by flow cytometric analysis using two staining panels. Panel 1 contained anti-CD19-BUV395, anti-CD38-BV421 (or BV785), anti-human IgG-PE (or APC), anti-CD138-APC (or PE), anti-IgD-BV605, anti-IgM-PerCP-C5.5, anti-CD3-BUV737, anti-CD27-BV510, anti-CD10-BV711, and LIVE/DEAD™ Near-IR fixable stain. Panel 2 contained anti-CD19-BUV395, anti-ICOS-PE, anti-CD40L-BV605, anti-CD4-PerCP-Cy5.5, anti-CD86-BV711 (or anti-CD19-BV711 in lieu of anti-CD19-BUV395), and anti-CD28-APC, and LIVE/DEAD™ Near-IR fixable stain. All antibodies were used at 1µg/ml with the following exceptions: anti-human IgG-PE and anti-human IgG-AF647 were used at 0.25 µg/ml. Anti-CD19-BUV395 was diluted 1:50 and anti-CD3-BUV737 was diluted to 1:40 into their respective stain cocktails. Cells were analyzed on an LSRII flow cytometer (BD Biosciences).

**[0720]** Inhibition of CD4+ T cell recovery and activation as demonstrated by the lack of CD40L and ICOS upregulation for exemplary WT, vIgD, or CTLA-4 stack proteins with TACI or BCMA are shown in **FIG. 8A** (CD4+ T cell recovery), **FIG**. **8B** (CD4+CD40L+ cell recovery), and **FIG. 8C** (CD4+ICOS+ cell recovery). In this example, all molecules contained the same CTLA-4 vIgD. Inhibition of T cell activity was only observed for molecules that contained a CTLA-4 IgSF domain, including by the CTLA-4-containing multidomain stack molecules containing TACI or BCMA TD. Similar levels of T cell inhibition were observed whether the particular CTLA-4 IgSF was used as a single domain or included as part of a multidomain stacked molecule.

**[0721]** Inhibition of total B cell recovery and B cell activation as demonstrated from the lack of CD86 upregulation for exemplary proteins are shown in **FIG. 8D** (CD19+ B cell recovery) and **FIG. 8E** (B cell activation/upregulation of CD86)**,** respectively. Inhibition of IgM secretion from B cells for exemplary proteins are shown in **FIG. 8F**. Inhibition of B cell activity was only observed for multidomain stack molecules containing a TACI vTD or a BCMA vTD.

## Example 11. Assessment of the Activity of Multi-Specific Constructs in an In Vivo KLH Immunization Model

**[0722]** This Example describes the assessment of exemplary tested multidomain stack proteins (described in Example 7 and Table E7) to affect immune responses to keyhole limpet hemocyanin (KLH) *in vivo* in mice. The mouse KLH immunization model can be used to evaluate the effects of the immunomodulatory molecules on antigen-specific responses to the T cell-dependent antigen KLH, following either one or two injections of KLH. Two injections of KLH, each separated by at least 7 days, provides a model that can test a secondary immune response in the period following the 2$^{nd}$ injection. This Example describes a study that evaluated the multidomain stack molecules, as compared to molar-

matched levels of an Fc isotype control protein and abatacept, in response to two injections of KLH (on Day 1 and Day 13). Activity of test articles observed in the mouse KLH model can often predict their immunomodulatory effects in humans.

**[0723]** To begin the KLH study, 10-week old female C57/BL6NJ mice (The Jackson Laboratories, Sacramento, CA) were randomized into 6 groups of 5 mice each and dosed with the test articles as outlined in **Table E12** via intraperitoneal (IP) injection (dosed on Days 0, 6 and 12). Mice were administered 0.25 mg KLH (EMD Millipore, Cat. 374825-25MG) via IP injection on Days 1 and 13. The original commercial stock solution of KLH was diluted 4-fold with Dulbecco's phosphate-buffered saline (DPBS). Three mice remained untreated/uninjected as naïve controls (Group 7).

**Table E12. Test Article Descriptions and Dose Regimen**

| Group # | # of Mice | Test Article(s) | Dose Level | Dose Volume (mL/kg) | Dose Schedule (D = Study Day) | Route of Delivery |
|---|---|---|---|---|---|---|
| 1 | 5 | Fc control | 0.1mg | 12.5 | D0,6,12 | IP |
| 2 | 5 | Abatacept | 0.17mg | 12.5 | D0,6,12 | IP |
| 3 | 5 | CTLA-4 186–GSG4S – Fc- (G4S)4 – BCMA 406 (SEQ ID NO: 601) | 0.20mg | 12.5 | D0,6,12 | IP |
| 4 | 5 | CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) | 0.20mg | 12.5 | D0,6,12 | IP |
| 5 | 5 | CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406 (SEQ ID NO: 602) | 0.20mg | 12.5 | D0,6,12 | IP |
| 6 | 5 | CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 611) | 0.20mg | 17.5 | D0,6,12 | IP |
| 7 | 3 | None (naïve controls) | N/A | N/A | N/A | N/A |

N/A = not applicable

**[0724]** On Day 19, all mice were anesthetized with isoflurane and blood collected into serum separator tubes. Mice were sacrificed, and their spleens removed, weighed, and placed into DPBS on ice. Whole blood was centrifuged, and the serum removed and stored at -80°C until analyzed for anti-KLH levels by enzyme-linked immunosorbent assay (ELISA). Spleens were processed to single cell suspensions, the red blood cells (RBC) lysed using RBC Lysis Buffer (Biolegend, Cat. 420301)

according to the manufacturer's instructions, and the cells counted in each sample using dual-fluorescence viability, using acridine orange/propidium iodide (AO/PI) staining (Nexcelom, Cat. CS2-0106-5mL).

**[0725]** Each spleen sample was then stained for flow cytometry analysis of immune cell subsets using the following method: 1 x $10^6$ live cells were placed into a well of two 96-well plates (Corning, Cat. 3797; one plate for a B cell-specific panel and one for a T cell-specific panel), centrifuged at 1500 x *g* for 10 seconds, the supernatant removed, and the cell pellet washed twice with DPBS. The pellets were resuspended in 100 µL of live-dead stain (LIVE/DEAD Fixable Aqua Dead Cell Stain Kit, Life Technologies Corp., 1:1000 dilution in DPBS) and incubated for 10 min in the dark at room temperature. Following two washes with flow cytometry buffer (175 µL each), tumor pellets were resuspended in Mouse BD Fc Block (diluted 1:50 with flow buffer), and incubated in the dark for an additional 5 min at RT. Without any additional washes, 50 µL of a cocktail of the following flow cytometry antibodies (diluted in flow cytometry buffer) were added to each well of cells for the B or T cell panels. For the B cell panel, the following antibodies were combined for the cocktail: anti-mouse CD19 BUV395 (clone 1D3, Becton-Dickinson; 1:100), anti-mouse CD138 BV421 (clone 281-2, BioLegend Inc.; 1:100, final concentration), anti-mouse CD3ε BV510 (clone 17A2, BioLegend Inc.; 1:100, final concentration), anti-mouse IgD BV605 (clone 11-26c.2a, BioLegend Inc.; 1:100, final concentration), anti-mouse B220 BV785 (clone RA3-6B2, BioLegend Inc.; 1:100, final concentration), anti-mouse CD95 FITC (clone SA367H8, BioLegend Inc.; 1:100, final concentration), anti-mouse CD23 PerCP Cy5.5 (clone B3B4, BioLegend Inc.; 1:100, final concentration), anti-mouse GL7 PE (clone GL7, BioLegend Inc.; 1:100, final concentration), anti-mouse Gr1 PE Cy7 (clone RB6-8C5, BioLegend Inc.; 1:100, final concentration), anti-mouse CD21 APC (clone 7E9, BioLegend Inc.; 1:100, final concentration), and anti-mouse IgM APC Cy7 (clone RMM-1, BioLegend Inc.; 1:100, final concentration). For the T cell panel, the following antibodies were combined for the cocktail: anti-mouse PD-1 BV421 (clone 29F.1A12, BioLegend Inc.; 1:100, final concentration), anti-mouse CD11b BV510 (clone M1/70, BioLegend Inc.; 1:100, final concentration), anti-mouse CD3ε BV605 (clone 145-2C11, BioLegend Inc.; 1:100, final concentration), anti-mouse CD8 BV785 (clone 53-6.7, BioLegend Inc.; 1:100, final concentration), anti-mouse CD44 FITC (clone IM7, BioLegend Inc.; 1:100, final concentration), anti-mouse CD4 PerCP Cy5.5 (clone GK1.5, BioLegend Inc.; 1:100, final concentration), anti-mouse CD62L PE (clone MEL-14, BioLegend Inc.; 1:100, final concentration), anti-mouse CXCR5 PE Dazzle (clone L138D7, BioLegend Inc.; 1:100, final concentration), anti-mouse CD25 PE Cy7 (clone PC61.5, BioLegend Inc.; 1:100, final concentration), and anti-mouse CD45 AF700 (clone 30-F11, BioLegend Inc.; 1:100, final concentration). The cells were incubated with one of the antibody cocktails in the dark, on ice, with gentle mixing for 45 min, followed by two washes with flow cytometry buffer (175 µL per wash). Cell pellets were resuspended in 200 µL flow cytometry buffer and collected on an LSRII flow cytometer. Data were

analyzed using FlowJo software version 10.2 (FlowJo LLC, USA) and graphed using GraphPad Prism software (Version 8.1.2). Key cellular subset identification analysis included: total B cells ($B220^+$ cells), marginal zone (MZ) B cells ($B220^+$, $CD19^+$, $CD23^-$, $CD21^{high}$, $IgM^{high}$ cells), germinal center (GC) B cells (B220+, CD19+, $GL7^+$, $CD95^+$ cells), T follicular helper (Tfh) cells ($CD45^+$, $CD3^+$, $CD4^+$, $PD1^+$, $CD185^+$ cells), $CD4^+$ T effector memory ($T_{em}$) cells ($CD45^+$, $CD3^+$, $CD4^+$, $CD44^+$, $CD62L^-$ cells), and $CD8^+$ $T_{em}$ cells ($CD45^+$, $CD3^+$, $CD8^+$, $CD44^+$, $CD62L^-$ cells).

**[0726]** Statistically significant differences ($p < 0.05$) between groups for all analyses were determined by one-way analysis of variance (ANOVA) or unpaired Student's t-test using GraphPad Prism software (Version 8.1.2).

**[0727]** To determine the extent to which the multidomain stack molecules inhibited KLH-mediated antibody immune responses compared to an Fc isotype control (SEQ ID NO:589), serum samples were evaluated for concentrations of anti-KLH antibodies by one of two ELISA assays. The ELISA assays measured either IgM- or IgG1-specific anti-KLH levels in the serum, and utilized assays in which mouse serum samples at numerous dilutions were incubated in plates coated with KLH, followed by washes and detection with 1:2000 goat anti-mouse IgG1:HRP or 1:5000 goat anti-mouse IgM:HRP. Color development was achieved using a TMB Substrate Kit (SeraCare) and the ELISA plates analyzed on a plate reader (EMax® Plus Microplate Reader, Molecular Devices LLC). There was no standard curve for the assay, thus optical density (OD) was used to compare the levels of anti-KLH antibodies; the higher the OD, the greater the levels of anti-KLH antibodies in the serum sample. For anti-KLH IgM OD levels, data are presented in **FIG. 9** and statistical analysis by 1-way ANOVA and Tukey's multiple comparison test presented in **Table E13**; anti-KLH IgG1 OD levels are presented in **FIG. 10** and statistical analysis by 1-way ANOVA and Tukey's multiple comparison test presented in **Table E14A**. Results demonstrate that each of the tested multidomain stack molecules were each able to significantly reduce anti-KLH IgM levels in serum compared to Fc control treatment, with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) demonstrating the biggest reductions amongst all test articles. Abatacept treatment had no effect on the levels of anti-KLH IgM levels in serum compared to Fc control treatment. Each of the multidomain stack molecules tested also were able to significantly reduce anti-KLH IgG1 levels compared to Fc control, with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) again demonstrating the greatest reductions. Abatacept was also able to significantly reduce the anti-KLH IgG1 levels in serum, but exemplary multidomain stack molecules were as effective or induced more dramatic reductions than abatacept (**FIG. 10**). These results indicate that these multidomain stack molecules are efficacious at reducing T cell-dependent antibody immune responses and do so as well as or better than abatacept, a commercially available and approved therapeutic for many autoimmune and inflammatory diseases.

| Table E13. Statistical Analysis anti-KLH IgM OD levels | |
|---|---|
| **Comparison** | **p-value** |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | <0.05 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | =0.0001 |
| Fc control vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | <0.01 |
| Fc control vs naïve | <0.001 |
| CTLA-4 186 GSG4S Fc (G4S)4 TACI 541<br>vs<br>CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406 | <0.0001 |
| CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406<br>vs<br>CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406 | =0.0115 |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406<br>vs<br>CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | =0.0035 |
| CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406<br>vs<br>naïve | <0.001 |

| Table E14A. Statistical Analysis anti-KLH IgG1 OD levels | |
|---|---|
| **Comparison** | **p-value** |
| Fc control vs abatacept | =0.0024 |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | =0.0044 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | =0.0006 |
| Fc control vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | =0.0017 |
| Fc control vs naïve | =0.0032 |

**[0728]** As shown in **FIG. 11**, mice treated with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) had significantly smaller spleens at the end of the study (Day 19) compared to Fc control-treated mice ($p < 0.001$ by t-test), as did mice treated with CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO:611) compared to Fc1.1-treated mice ($p = 0.01$ by t-test). Mice treated with the multidomain stack molecule CTLA-4 186– GSG4S – Fc- (G4S)4 – BCMA 406 (SEQ ID NO:601) tended to have smaller spleens compared to Fc1.1-treated mice, but the difference was not quite statistically different ($p = 0.087$ by t-test). There were no differences between the Fc isotype control-treated mice and

the naïve mice or mice treated with abatacept or CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406 (SEQ ID NO: 602, which contained the same BCMA vTD as the multidomain stack molecule in SEQ ID NO:601, but contained a WT CTLA-4 IgD instead of a vIgD. The smaller spleens are indicative of reductions in lymphocytes, which can have immunomodulatory effects on the pathogenesis of autoimmune and inflammatory diseases associated with heightened immune responses, particularly those driven by B and/or T cells. Statistical analysis of spleen weights is shown in in Table E14B.

**Table E14B. Statistical Analysis of Spleen Weights**

| Spleen weight | p-value |
|---|---|
| Fc control vs. CTLA4 186 GSG4S Fc1.1 (G4S)4 TACI 541 | 0.0040 |
| Abatacept vs. CTLA4 186– GSG4S – Fc1.1- (G4S)4 – BCMA 406 | 0.0025 |
| Abatacept vs. CTLA4 186 GSG4S Fc1.1 (G4S)4 TACI 541 | <0.0001 |
| Abatacept vs. CTLA4 WT ECD 1 GSG4S Fc1.1 (G4S)4 TACI 541 | 0.0021 |

**[0729]** Along these lines, of particular importance to the pathogenesis of autoimmune and inflammatory diseases are cell types that promote B cell survival and differentiation, antibody production, and T cell effector memory. These cell types include, but are not limited to, the following: B cells, marginal zone (MZ) B cells, germinal center (GC) B cells, T follicular helper (Tfh) cells, and $CD4^+$ and $CD8^+$ T effector memory ($T_{em}$) cells. Therapeutics whose mechanisms of action include reducing these cell types would be anticipated to be efficacious in the treatment of numerous autoimmune and inflammatory diseases. As shown in **FIG 12A-12H**, certain tested multidomain stack molecules were able to reduce the percentage and number per spleen of B cells, MZ B cells, GC B cells, and Tfh as compared to the Fc isotype control (SEQ ID NO:589), with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) being the most potent of the variants, followed by CTLA-4 186– GSG4S – Fc-(G4S)4 – BCMA 406 (SEQ ID NO:601), and then CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO:611) containing a WT CTLA-4 IgD and a TACI vTD. These multidomain stack molecules were as effective or better than abatacept in their ability to reduce the percentage or numbers of these populations that are important in B cell survival and differentiation and antibody production. Statistical analysis of the exemplary multidomain stack molecules compared to Fc control or abatacept is shown in **Table E15**.

| **Table E15. Statistical Analysis** | |
|---|---|
| **Comparison** | ***p*-value** |
| **B220+ cells as a % of live cells** | |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | <0.0001 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | <0.0001 |
| Fc control vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406 | 0.0344 |
| Fc control vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | <0.0001 |
| Abatacept vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | <0.0001 |
| Abatacept vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | <0.0001 |
| Abatacept vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | 0.0002 |
| **Marginal zone (MZ) B cells as a % of B cells** | |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0082 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | <0.0001 |
| Fc control vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | 0.0007 |
| Abatacept vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0001 |
| Abatacept vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | <0.0001 |
| Abatacept vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | <0.0001 |
| **Germinal center B cells as % of live B cells** | |
| Fc control vs Abatecept | 0.0065 |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0085 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0016 |
| Fc control vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | 0.0663 |

| **Table E15. Statistical Analysis** | |
|---|---|
| **Comparison** | ***p*-value** |
| Abatacept vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406 | 0.0006 |
| **CD4+ T follicular helper (Tfh) cells as a % of CD4+ cells** | |
| Fc control vs Abatacept | 0.0341 |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0005 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | <0.0001 |
| Fc control vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | 0.0018 |
| Abatacept vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0065 |
| **B220+ cell # per spleen ($x10^6$)** | |
| Fc control vs Abatacept | 0.0092 |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.022 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0028 |
| Fc control vs naïve | 0.0162 |
| Abatacept vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | <0.0001 |
| Abatacept vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | <0.0001 |
| Abatacept vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | 0.0001 |
| **Marginal zona (MZ) cell # per spleen ($x10^6$)** | |
| Fc control vs Abatacept | 0.0009 |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0312 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0028 |

| **Table E15. Statistical Analysis** | |
|---|---|
| **Comparison** | ***p*-value** |
| Fc control vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | 0.0385 |
| Fc control vs naïve | 0.0044 |
| Abatacept vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | <0.0001 |
| Abatacept vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | <0.0001 |
| Abatacept vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406 | 0.0057 |
| Abatacept vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 TACI 541 | <0.0001 |
| **Germinal center (GC) B cell # per spleen ($x10^6$)** | |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0246 |
| Abatacept vs CTLA-4 WT ECD 1 GSG4S Fc (G4S)4 BCMA 406 | 0.0161 |
| CD4+ T follicular helper (Tfh) cell # per spleen ($x10^6$) | |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0376 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0083 |
| Abatacept vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0268 |
| Abatacept vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0057 |

**[0730]** As shown in **FIGS 13A-13D**, multidomain stack molecules containing a CTLA-4 vIgD and a TACI or BCMA vTD were able to reduce the percentage and number per spleen of $CD4^+$ and $CD8^+$ $T_{em}$ cells as compared to the Fc isotype control and abatacept. Thus, these results show that exemplary tested multidomain stack molecules were at least as effective, and often more effective than, abatacept in their ability to reduce the percentage or numbers of these important effector memory populations in the spleen. Statistical analysis of the exemplary multidomain stack molecules compared to Fc control or abatacept is shown in **Table E16**.

| **Table E16. Statistical Analysis** | |
|---|---|
| **Comparison** | ***p*-value** |
| **CD4+ Tem cells as a % of CD4+ T cells** | |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0035 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | <0.0001 |
| Abatacept vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0392 |
| Abatacept vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0004 |
| **CD8+ Tem cells as a % of CD8+ T cells** | |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0393 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0042 |
| Fc control vs naïve | 0.0072 |
| Abatacept vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0348 |
| Abatacept vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0037 |
| Abatacept vs naïve | 0.0064 |
| **CD4+ Tem cells # per spleen ($x10^6$)** | |
| Fc control vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0771 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0172 |
| Abatacept vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0038 |
| Abatacept vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0006 |
| **CD8+ Tem cells # per spleen ($x10^6$)** | |
| Fc control vs Abatacept | 0.0135 |
| Fc control vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0866 |
| Abatacept vs CTLA-4 186– GSG4S – Fc- (G4S)4 BCMA 406 | 0.0002 |
| Abatacept vs CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 | 0.0002 |

| Table E16. Statistical Analysis | |
|---|---|
| **Comparison** | ***p*-value** |
| Abatacept vs naïve | 0.0028 |

**[0731]** Together, these results indicate that multidomain stack molecules that inhibit both T cell activity and B cell activity can reduce immune responses and cell subset changes mediated by the T cell-dependent antigen KLH *in vivo* (i.e. anti-KLH levels in serum and changes in immune cell subsets). These results are consistent with the use of the CTLA-4 and BCMA/TACI multidomain stack proteins as efficacious therapeutics in the treatment of autoimmune and inflammatory diseases in which hyperactive lymphocytes play a role.

**Example 12. Assessment of the Activity of Multi-Specific Constructs in an In Vivo Mouse Lupus Model.**

**[0732]** This Example describes the assessment of exemplary CTLA-4 vIgD-Fc molecules, and an exemplary multidomain stack molecule containing TACI vTD with CTLA-4 vIgD domains (described in Example 7 and Table E7) to affect immune responses in an *in vivo* murine (NZB/NZW)F1 spontaneous lupus model. (NZBxNZW)F1 mice spontaneously develop an autoimmune disease very similar to human SLE and are regarded as one of the best mouse models of this disease. (NZB/NZW)F1 mice have high circulating concentrations of anti-dsDNA antibodies starting around 20 weeks of age, with the first clinical signs of disease detectable around 23 weeks of age. The mice develop hemolytic anemia, proteinuria, and progressive glomerulonephritis mediated by immune complex deposition in the glomerular basement membrane.

**[0733]** (NZB/NZW)F1 mice were dosed twice weekly via intraperitoneal (IP) injection with 14 mg/kg Fc control, or molar-matched amounts of CTLA-4 vIgD-Fc (186 CTLA-4-Fc) (21 mg/kg), or an exemplary multidomain stack Fc fusion molecule containing TACI vTD and CTLA-4 vIgD ('CTLA-4 vIgD-Fc-TACI vTD') (CTLA-4 186 GSG4S Fc (G4S)4 TACI 541) (25 mg/kg). Treatment started at group assignment (Week 22 of age) and continued through the end of the study. The study ended when mice reached Week 43 of age, though some animals were euthanized earlier in the study when they became moribund.

**[0734]** At various time points between 20 and 40 weeks of age, urine and serum samples were collected. Starting when mice were 20 weeks old, the concentration of protein in the urine from all mice on study was determined weekly with urinalysis test strips (Roche Chemstrip 2 GP, cat. 11895397160). Mean proteinuria scores over time in each treatment group are presented in **FIG. 14A**, and the mean percent change in body weight (weight loss is associated with advancing disease) in each group in plotted

in **FIG. 14B**. The percent survival of mice in each treatment group is plotted in **FIG. 14C**. Anti-double stranded (ds) DNA IgG serum titers were measured by Hooke Laboratories, Inc. (Lawrence, MA) using their in-house kit, and the results are presented in **FIG.14D**. Blood urea nitrogen (BUN) levels increase in these mice with advancing disease. BUN levels at termination of the study (or at sacrifice of mice that succumbed early) for each treatment group are shown in **FIG. 14E**. Statistical analysis was performed using Uncorrected Dunn's test, ** denotes p=0.0047 and p=0.0065; *** denotes p=0.0004).

**[0735]** Kidneys were collected at termination from each mouse and analyzed histologically in replicate Periodic acid-Schiff (PAS)-stained sections using the criteria described in Alperovich G et al, 2007. Lupus 16:18-24. All kidney sections were analyzed blind, by a pathologist unaware of the treatments and clinical scores. Glomerular lesions (mesangial expansion, endocapillary proliferation, glomerular deposits, and extracapillary proliferation) and tubular/interstitial lesions (interstitial infiltrates, tubular atrophy, and interstitial fibrosis) were analyzed and graded semi-quantitatively using a scoring system from 0 to 3, with 0=no changes, 1=mild changes, 2=moderate changes, and 3=severe changes. A total histological score for each mouse was calculated as the sum of the individual scores (maximum total score is 21). Kidney scores for total glomerular lesions, total tubular and interstitial lesions, and total kidney lesions are shown in **FIG. 14F**; as compared to Fc control treated mice, significantly improved renal histopathology was observed in animals treated with CTLA-4 vIgD-Fc ($p$=0.0068 vs. Fc group for total kidney lesions), or CTLA-4 vIgD-Fc-TACI vTD ($p$<0.0001 vs. Fc group).

**[0736]** For **FIG. 14G-14H**, the right kidney was collected from each mouse at study termination, weighed, dissected transversally, and frozen in a single optimal cutting temperature compound (OCT) block, before sectioning and immunohistochemical (IHC) staining of mouse IgG and mouse complement C3 to assess glomerular IgG and C3 deposition, respectively. The kidney sections were permeabilized with acetone and stained with FITC-conjugated rat monoclonal anti-mouse complement component C3 (Cedarlane) diluted 1:25 in Primary Antibody Diluent (Leica Biosystems), or AF594-conjugated goat anti-mouse IgG (Thermo Fisher Scientific) diluted 1:200 in Primary Antibody Diluent. Glomerular depositions of IgG and C3 were analyzed by a pathologist using a semiquantitative scoring system from 0 to 4, with 0=no deposits, 1=mild mesangial deposition, 2=marked mesangial deposition, 3=mesangial and slight capillary deposition, and 4=intense mesangial and mesangiocapillary deposition, based on the method described in Kelkka et al. (2014) *Antioxid Redox Signal.* 21:2231-45. As compared to Fc control treated mice, significantly reduced glomerular IgG and C3 were observed in animals treated with 186 CTLA-4 vIgD-Fc ($p$=0.0084 vs. Fc control group for IgG deposits, and $p$=0.0007 for C3 deposits), or CTLA-4 186 – GSG4S – Fc (G4S)4 – TACI 541 ($p$<0.0001 vs. Fc control group for IgG, and $p$=0.0053 for C3); data were analyzed for statistically significant differences using Kruskal-Wallis (non-parametric) test followed by uncorrected Dunn's multiple comparison test.

**[0737]** Results demonstrate that the CTLA-4 vIgD alone or a multi-domain immunomodulatory Fc protein containing a CTLA-4 vIgD and TACI vTD were each able to significantly suppress proteinuria, preserve body weight, enhance overall survival, reduce anti-dsDNA autoantibodies and BUN, reduce IgG and C3 renal deposits, and prevent or improve kidney disease in the (NZB/NZW)F1 mouse model of SLE. Exemplary molecules were also capable of potently reducing B and T cell subsets including plasma cells, follicular T helper cells, germinal center cells, and memory T cells in the spleens and lymph nodes of these mice (data not shown).

## Example 13: Comparative Evaluation of Multi-Specific Constructs in an In Vivo KLH Immunization Model

**[0738]** This Example describes the assessment of exemplary tested single domain Fc fusion proteins and one multidomain stack protein (described in **Example 6**, **Example 7** and **Table E7**) to affect immune responses to keyhole limpet hemocyanin (KLH) *in vivo* in mice. The mouse KLH immunization model can be used to evaluate the effects of the immunomodulatory molecules on antigen-specific responses to the T cell-dependent antigen KLH, following either one or two injections of KLH. Two injections of KLH, each separated by at least 7 days, provides a model that can evaluate both a primary immune response following the 1st KLH injection, and a secondary immune response in the period following the 2nd injection. This Example describes a study that evaluated the activity of multiple BCMA single domain-containing molecules, such as soluble wild-type (WT) or variant BCMA vTDs formatted as Fc fusions, as well as the multidomain stack molecule CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541, in response to two injections of KLH without adjuvant (on Study Day 0 and Day 12). These test articles were compared to administration of molar-matched levels of an Fc isotype control protein or abatacept (WT CTLA-4-Fc). Activity of test articles observed in the mouse KLH model can often predict their immunomodulatory effects in humans.

**[0739]** To begin the KLH study, 10-week old female C57/BL6NJ mice (The Jackson Laboratories, Sacramento, CA) were randomized into 12 groups of 5 mice each. Mice were administered 0.25 mg KLH (EMD Millipore, Cat. 374825-25MG) via intraperitoneal (IP) injection on Days 0 and 12; the original commercial stock solution of KLH was diluted to the appropriate concentration with Dulbecco's phosphate-buffered saline (DPBS) prior to injection. Mice were dosed with the test articles as outlined in **Table E17** via IP injection (dosed on Days 4 and 11). Six mice remained untreated/uninjected as naïve controls (Group 13). Serum was collected on Day 5 (24 hr post-1st dose), Day 12 (24 hr post-2nd dose/pre-KLH boost), and Day 20 to evaluate drug exposure, ADA, and/or anti-KLH antibody levels. One animal in Group 10 received an incomplete dose of test article and was therefore removed from the study.

| Table E17. Test Article Descriptions and Dose Regimen | | | | | | |
|---|---|---|---|---|---|---|
| **Group #** | **# of Mice** | **Test Article(s)** | **Dose Level (mg/dose)** | **(mg/kg)** | **Dose Schedule (D = Study Day)** | **Route of Delivery** |
| 1 | 5 | Fc control | 0.225 | 11.3 | D4 and D11 | IP |
| 2 | 5 | abatacept (WT hCTLA-4-Fc) | 0.342 | 17.1 | D4 and D11 | IP |
| 3 | 5 | 186 CTLA-4 vIgD-Fc | 0.346 | 17.3 | D4 and D11 | IP |
| 4 | 5 | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.400 | 20 | D4 and D11 | IP |
| 5 | 5 | TACI 30-110 – Fc | 0.306 | 15.3 | D4 and D11 | IP |
| 6 | 5 | TACI 13-118 – Fc | 0.327 | 16.4 | D4 and D11 | IP |
| 10 | 4 | 406 BCMA -Fc | 0.278 | 13.9 | D4 and D11 | IP |
| 11 | 5 | 381 BCMA -Fc | 0.278 | 13.9 | D4 and D11 | IP |
| 12 | 5 | 411 BCMA -Fc | 0.279 | 13.9 | D4 and D11 | IP |
| 13 | 6 | None (naïve) | N/A | N/A | N/A | N/A |

N/A = not applicable

**[0740]** On Day 20, all mice were anesthetized with isoflurane and blood collected into serum separator tubes. Mice were sacrificed, and their spleens removed, weighed, and placed into DPBS on ice. Whole blood was centrifuged, and the serum removed and stored at -80°C until analyzed for anti-KLH levels by enzyme-linked immunosorbent assay (ELISA). Spleens were processed to single cell suspensions, the red blood cells (RBC) lysed using RBC Lysis Buffer (Biolegend, Cat. 420301) according to the manufacturer's instructions, and the cells counted in each sample using dual-fluorescence viability, using acridine orange/propidium iodide (AO/PI) staining (Nexcelom, Cat. CS2-0106-5mL).

**[0741]** Each spleen sample was then stained for flow cytometry analysis of immune cell subsets using the following method: 1 x $10^6$ live cells were placed into a well of two 96-well plates (Corning, Cat. 3797; one plate for a B cell-specific panel and one for a T cell-specific panel), centrifuged at 1500 x *g* for 10 seconds, the supernatant removed, and the cell pellet washed twice with DPBS. The pellets were resuspended in 100 µL of live-dead stain (LIVE/DEAD Fixable Aqua Dead Cell Stain Kit, Life Technologies Corp., 1:1000 dilution in DPBS) and incubated for 10 min in the dark at room temperature. Following two washes with flow cytometry buffer (175 µL each), tumor pellets were resuspended in Mouse BD Fc Block (diluted 1:50 with flow buffer), and incubated in the dark for an additional 5 min at

RT. Without any additional washes, 50 µL of a cocktail of the following flow cytometry antibodies (diluted in flow cytometry buffer) were added to each well of cells for the B or T cell panels. For the B cell panel, the following antibodies were combined for the cocktail: anti-mouse CD19 BUV395 (clone 1D3, Becton-Dickinson; 1:100), anti-mouse CD138 BV421 (clone 281-2, BioLegend Inc.; 1:100, final concentration), anti-mouse CD3ε BV510 (clone 17A2, BioLegend Inc.; 1:100, final concentration), anti-mouse IgD BV605 (clone 11-26c.2a, BioLegend Inc.; 1:100, final concentration), anti-mouse B220 BV785 (clone RA3-6B2, BioLegend Inc.; 1:100, final concentration), anti-mouse CD95 FITC (clone SA367H8, BioLegend Inc.; 1:100, final concentration), anti-mouse CD23 PerCP Cy5.5 (clone B3B4, BioLegend Inc.; 1:100, final concentration), anti-mouse GL7 PE (clone GL7, BioLegend Inc.; 1:100, final concentration), anti-mouse Gr1 PE Cy7 (clone RB6-8C5, BioLegend Inc.; 1:100, final concentration), anti-mouse CD21 APC (clone 7E9, BioLegend Inc.; 1:100, final concentration), and anti-mouse IgM APC Cy7 (clone RMM-1, BioLegend Inc.; 1:100, final concentration). For the T cell panel, the following antibodies were combined for the cocktail: anti-mouse PD-1 BV421 (clone 29F.1A12, BioLegend Inc.; 1:100, final concentration), anti-mouse CD11b BV510 (clone M1/70, BioLegend Inc.; 1:100, final concentration), anti-mouse CD3ε BV605 (clone 145-2C11, BioLegend Inc.; 1:100, final concentration), anti-mouse CD8 BV785 (clone 53-6.7, BioLegend Inc.; 1:100, final concentration), anti-mouse CD44 FITC (clone IM7, BioLegend Inc.; 1:100, final concentration), anti-mouse CD4 PerCP Cy5.5 (clone GK1.5, BioLegend Inc.; 1:100, final concentration), anti-mouse CD62L PE (clone MEL-14, BioLegend Inc.; 1:100, final concentration), anti-mouse CXCR5 PE Dazzle (clone L138D7, BioLegend Inc.; 1:100, final concentration), anti-mouse CD25 PE Cy7 (clone PC61.5, BioLegend Inc.; 1:100, final concentration), and anti-mouse CD45 AF700 (clone 30-F11, BioLegend Inc.; 1:100, final concentration). The cells were incubated with one of the antibody cocktails in the dark, on ice, with gentle mixing for 45 min, followed by two washes with flow cytometry buffer (175 µL per wash). Cell pellets were resuspended in 200 µL flow cytometry buffer and collected on an LSRII flow cytometer. Data were analyzed using FlowJo software version 10.2 (FlowJo LLC, USA) and graphed using GraphPad Prism software (Version 8.1.2). Key cellular subset identification analysis included: total B cells ($B220^+$ cells), marginal zone (MZ) B cells ($B220^+$, $CD19^+$, $CD23^-$, $CD21^{high}$, $IgM^{high}$ cells), germinal center (GC) B cells ($B220^+$, $CD19^+$, $GL7^+$, $CD95^+$ cells), T follicular helper (Tfh) cells ($CD45^+$, $CD3^+$, $CD4^+$, $PD\text{-}1^+$, $CD185^+$ cells), $CD4^+$ T effector memory ($T_{em}$) cells ($CD45^+$, $CD3^+$, $CD4^+$, $CD44^+$, $CD62L^-$ cells), and $CD8^+$ $T_{em}$ cells ($CD45^+$, $CD3^+$, $CD8^+$, $CD44^+$, $CD62L^-$ cells).

**[0742]** Statistically significant differences ($p < 0.05$) between groups were determined by one-way analysis of variance (ANOVA) and uncorrected Fisher's Least Significant Difference (LSD) multiple comparison test using GraphPad Prism software (Version 8.1.2).

**[0743]** To determine the extent to which the test articles inhibited KLH-mediated antibody immune responses compared to an Fc isotype control (SEQ ID NO:589), serum samples were evaluated for

concentrations of anti-KLH antibodies in two ELISA assays. The ELISA assays measured either IgM- or IgG1-specific anti-KLH levels in the serum. Mouse serum samples at numerous dilutions were incubated in plates coated with KLH, followed by washes and detection with 1:2000 goat anti-mouse IgG1:HRP or 1:5000 goat anti-mouse IgM:HRP. Color development was achieved using a TMB Substrate Kit (SeraCare) and the ELISA plates analyzed on a plate reader (SpectraMax® iD3 Microplate Reader, Molecular Devices LLC). There was no standard curve for the assay, thus optical density (OD) was used to compare the levels of anti-KLH antibodies; the higher the OD, the greater the levels of anti-KLH antibodies in the serum sample. For anti-KLH IgM OD levels, data are presented in **FIG. 18A** (primary response), **FIG. 18B** (secondary response) and statistical analysis by 1-way ANOVA and uncorrected Fisher's LSD multiple comparison test presented in **Table E18** and **Table E19**, respectively. Anti-KLH IgG1 OD levels are presented in **FIG. 18C** (primary response), **FIG. 18D** (secondary response) and statistical analysis by 1-way ANOVA and uncorrected Fisher's LSD multiple comparison test presented in **Tables E18-E21.** Results demonstrate that each of the test articles were able to significantly reduce anti-KLH IgM levels in serum during the primary immune response compared to Fc control treatment, with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) demonstrating the largest reductions amongst all test articles, and abatacept treatment having the most modest effect (**FIG. 18A)**. For the secondary response on Day 20, measured 9 days after the 2$^{nd}$ and last dose of test article, all test articles except TACI 13-118-Fc and 406 BCMA -Fc induced significant reductions in anti-KLH IgM levels, with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) demonstrating the largest reductions (**FIG. 18B**). Each of the test articles were also able to significantly reduce anti-KLH IgG1 levels during the primary immune response compared to Fc control, with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) again demonstrating the greatest reductions (**FIG. 18C**). For the secondary response to KLH, all test articles except TACI 30-110-Fc, TACI 13-118-Fc, and 411 BCMA -Fc significantly reduced levels of anti KLH IgG1 (**FIG. 18D**). These results indicate that most of the molecules containing the BCMA vTD were efficacious at reducing the T cell-dependent antibody immune response to KLH, with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610), and 381 BMCA -Fc exhibiting the most significant effects in this mouse immunization model.

**Table E18. Statistical Analysis of anti-KLH IgM OD levels (primary response; FIG. 18A)**

| Comparison | p-value | Significant? |
|---|---|---|
| Fc Control vs. abatacept | 0.0167 | Yes |
| Fc Control vs. 186 CTLA-4 vIgD-Fc | <0.0001 | Yes |
| Fc Control vs. CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | Yes |

| **Table E18. Statistical Analysis of anti-KLH IgM OD levels (primary response; FIG. 18A)** | | |
|---|---|---|
| **Comparison** | **p-value** | **Significant?** |
| Fc Control vs. TACI 30-110 – Fc | <0.0001 | Yes |
| Fc Control vs. TACI 13-118 – Fc | <0.0001 | Yes |
| Fc Control vs. 406 BCMA -Fc | <0.0001 | Yes |
| Fc Control vs. 381 BCMA -Fc | <0.0001 | Yes |
| Fc Control vs. 411 BCMA -Fc | <0.0001 | Yes |
| Fc Control vs. Naive | <0.0001 | Yes |

| **Table E19. Statistical Analysis of anti-KLH IgM OD levels (secondary response; FIG. 18B)** | | |
|---|---|---|
| **Comparison** | **p-value** | **Significant?** |
| Fc Control vs. abatacept | <0.0001 | Yes |
| Fc Control vs. 186 CTLA-4 vIgD-Fc | <0.0001 | Yes |
| Fc Control vs. CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | Yes |
| Fc Control vs. TACI 30-110 – Fc | 0.0283 | Yes |
| Fc Control vs. TACI 13-118 – Fc | 0.4653 | No |
| Fc Control vs. 406 BCMA -Fc | 0.0971 | No |
| Fc Control vs. 381 BCMA -Fc | 0.0032 | Yes |
| Fc Control vs. 411 BCMA -Fc | 0.0218 | Yes |
| Fc Control vs. Naive | <0.0001 | Yes |

| **Table E20. Statistical Analysis of anti-KLH IgG1 OD levels (primary response; FIG. 18C)** | | |
|---|---|---|
| **Comparison** | **p-value** | **Significant?** |
| Fc Control vs. abatacept | 0.0457 | Yes |
| Fc Control vs. 186 CTLA-4 vIgD-Fc | 0.0057 | Yes |
| Fc Control vs. CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | Yes |
| Fc Control vs. TACI 30-110 – Fc | 0.0218 | Yes |
| Fc Control vs. TACI 13-118 – Fc | 0.0093 | Yes |
| Fc Control vs. 406 BCMA -Fc | 0.0039 | Yes |

| **Table E20. Statistical Analysis of anti-KLH IgG1 OD levels (primary response; FIG. 18C)** | | |
|---|---|---|
| **Comparison** | **p-value** | **Significant?** |
| Fc Control vs. 381 BCMA -Fc | 0.0006 | Yes |
| Fc Control vs. 411 BCMA -Fc | 0.0002 | Yes |
| Fc Control vs. Naive | <0.0001 | Yes |

| **Table E21. Statistical Analysis of anti-KLH IgG1 OD levels (secondary response; FIG. 18D)** | | |
|---|---|---|
| **Comparison** | **p-value** | **Significant?** |
| Fc Control vs. abatacept | <0.0001 | Yes |
| Fc Control vs. 186 CTLA-4 vIgD-Fc | <0.0001 | Yes |
| Fc Control vs. CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | Yes |
| Fc Control vs. TACI 30-110 – Fc | 0.5367 | No |
| Fc Control vs. TACI 13-118 – Fc | 0.1477 | No |
| Fc Control vs. 406 BCMA -Fc | 0.0397 | Yes |
| Fc Control vs. 381 BCMA -Fc | 0.0286 | Yes |
| Fc Control vs. 411 BCMA -Fc | 0.8374 | No |
| Fc Control vs. Naive | <0.0001 | Yes |

**[0744]** As shown in **FIG. 19A and 19B**, mice treated with all test articles except TACI 30-110-Fc or TACI 13-118-Fc had significantly smaller spleens as assessed by weight and cell number, respectively, at the end of the study (Day 20) compared to Fc control-treated mice (**Table E22**), and mice treated with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) had the smallest spleens of all the treatment groups. Mice treated with each of the test articles except 186 CTLA-4 vIgD-Fc also had significantly fewer spleen cells than the Fc control group, and the mice treated with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) had the lowest number of splenocytes among all treatment groups. The smaller spleens are indicative of reductions in lymphocytes, which can have immunomodulatory effects on the pathogenesis of autoimmune and inflammatory diseases associated with heightened immune responses, particularly those driven by B and/or T cells. Statistical analyses of spleen weights and total cell numbers are shown in **Table E22** and **Table E23**, respectively.

| **Table E22. Statistical Comparisons Across All Treatment Groups for Spleen Weights (FIG. 19A):** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA -Fc | 411 BCMA -Fc |
| abatacept | 0.0084 | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | 0.0056 | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | 0.0033 | 0.0124 | | | | | | |
| TACI 30-110 – Fc | ns | 0.0228 | 0.0148 | <0.0001 | | | | | |
| TACI 13-118 – Fc | ns | 0.0415 | 0.0267 | <0.0001 | ns | | | | |
| 406 BCMA -Fc | 0.0253 | ns | ns | 0.0009 | ns | ns | | | |
| 381 BCMA -Fc | 0.0101 | ns | ns | 0.0027 | 0.0269 | 0.0483 | ns | | |
| 411 BCMA -Fc | 0.0022 | ns | ns | 0.0122 | 0.0067 | 0.013 | ns | ns | |
| Naive | 0.041 | ns | ns | 0.0002 | ns | ns | ns | ns | ns |

| **Table E23. Statistical Comparisons Across All Treatment Groups for Splenic Cell Numbers (FIG. 19B)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA - Fc | 381 BCMA -Fc | 411 BCMA -Fc |
| abatacept | 0.0168 | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | ns | ns | | | | | | | |

| **Table E23. Statistical Comparisons Across All Treatment Groups for Splenic Cell Numbers (FIG. 19B)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA - Fc | 381 BCMA -Fc | 411 BCMA -Fc |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | <0.001 | <0.0001 | | | | | | |
| TACI 30-110 – Fc | 0.0022 | ns | ns | <0.0001 | | | | | |
| TACI 13-118 – Fc | 0.0079 | ns | ns | <0.0001 | ns | | | | |
| 406 BCMA -Fc | <0.0001 | ns | 0.0174 | 0.0006 | ns | ns | | | |
| 381 BCMA -Fc | <0.0001 | 0.0011 | 0.0003 | 0.017 | 0.0094 | 0.0027 | ns | | |
| 411 BCMA -Fc | <0.0001 | 0.0024 | 0.0005 | 0.009 | 0.0176 | 0.0054 | ns | ns | |
| Naive | <0.0001 | 0.0112 | 0.0025 | 0.0009 | ns | 0.0241 | ns | ns | ns |

**[0745]** Of particular importance to the pathogenesis of autoimmune and inflammatory diseases are cell types that promote B cell survival and differentiation, antibody production, and T cell effector memory. These cell types include, but are not limited to, the following: total B cells, marginal zone (MZ) B cells, germinal center (GC) B cells, T follicular helper (Tfh) cells, and $CD4^+$ and $CD8^+$ T effector memory ($T_{em}$) cells. Therapeutics whose mechanisms of action include reducing these cell types would be anticipated to be efficacious in the treatment of numerous autoantibody-mediated diseases. Treatment with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610), or any of the BCMA -Fc test articles substantially reduced the numbers of multiple splenic B cell subsets compared to the remaining treatment groups, including impacts on transitional-2 ($B220^+$ $CD19^+$ $CD23^+$ $CD21^{high}$ $IgM^{high}$), follicular ($B220^+$ $CD19^+$ $CD23^+$ $CD21^+$ $IgM^+$), marginal zone ($B220^+$ $CD19^+$ $CD23^{neg}$ $CD21^{high}$ $IgM^{high}$), germinal centre ($B220^+$ $CD19^+$ $GL7^+$ $CD95^+$), and plasma cells ($B220^{low}$ $CD19^+$ $CD138^{high}$) (**FIG. 20** and **FIG 21**).

These BCMA vTD-containing single or TACI vTD- or BCMA vTD-containing multi-domain molecules were as effective or better than abatacept or the two WT TACI -Fc molecules (TACI 13-188-Fc and TACI 30-110-Fc) in their ability to reduce the percentage (not shown) or numbers of these populations that are important in B cell survival and differentiation and antibody production. Statistical analyses from flow cytometry data of Day 20 splenocytes are shown in **Tables E24-E42.**

**[0746]** The splenic CD3+, CD4+, or CD8+ T cell populations were largely unaffected by the BCMA vTD-containing test articles (Groups 7-12), though treatment of the mice with CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) (Group 4) did reduce the numbers of these T cell populations compared to the Fc control group (**FIG. 22A-22C**). CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610) also reduced Tcm and Tem memory T cells compared to the Fc control group, while the TACI or BCMA single domain test articles did not (**FIG. 23**). As compared to the Fc control, all of the test articles reduced the numbers of follicular helper T cells ($CD45^+$, $CD3^+$, $CD4^+$, $PD\text{-}1^+$, $CD185^+$), which interact with B cells in the germinal center and are important contributors to T cell-dependent antibody responses (**FIG. 22D**).

**Table E24. Statistical Analysis of Splenic B Cell Subsets—Cell Numbers vs. Fc Control Group (FIG. 20)**

| Comparison | T1 B cells | T2 B cells | Follic B cells | Marginal Zone B cells | GC B cells | Plasma Cells |
|---|---|---|---|---|---|---|
| Fc Control vs. abatacept | 0.4773 | 0.0760 | 0.0009 | 0.0022 | <0.0001 | 0.0014 |
| Fc Control vs. 186 CTLA-4 vIgD-Fc | 0.5970 | 0.1130 | 0.0189 | 0.0003 | <0.0001 | 0.0580 |
| Fc Control vs. CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.0345 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Fc Control vs. TACI 30-110 – Fc | 0.2738 | 0.4820 | <0.0001 | <0.0001 | 0.0152 | <0.0001 |
| Fc Control vs. TACI 13-118 – Fc | 0.5942 | 0.0045 | <0.0001 | <0.0001 | 0.0115 | 0.0012 |
| Fc Control vs. 406 BCMA -Fc | 0.9686 | <0.0001 | <0.0001 | <0.0001 | 0.0030 | <0.0001 |
| Fc Control vs. 381 BCMA -Fc | 0.5743 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Fc Control vs. 411 BCMA -Fc | 0.4039 | <0.0001 | <0.0001 | <0.0001 | 0.0004 | <0.0001 |

| Table E24. Statistical Analysis of Splenic B Cell Subsets—Cell Numbers vs. Fc Control Group (FIG. 20) | | | | | | |
|---|---|---|---|---|---|---|
| **Comparison** | **T1 B cells** | **T2 B cells** | **Follic B cells** | **Marginal Zone B cells** | **GC B cells** | **Plasma Cells** |
| Fc Control vs. Naive | 0.2333 | 0.0241 | | <0.0001 | <0.0001 | <0.0001 |

| Table E25. Statistical Analysis of Splenic T Cell Subsets—Cell Numbers vs. Fc Control Group (FIG. 22A-22D) | | | | |
|---|---|---|---|---|
| **Comparison** | **CD3+ T cells** | **CD8+ T cells** | **CD4+ T cells** | **CD4+ Tfh cells** |
| Fc Control vs. abatacept | 0.1393 | 0.2757 | 0.0800 | <0.0001 |
| Fc Control vs. 186 CTLA-4 vIgD-Fc | 0.4956 | 0.7235 | 0.2681 | <0.0001 |
| Fc Control vs. CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.0001 | 0.0034 | <0.0001 | <0.0001 |
| Fc Control vs. TACI 30-110 – Fc | 0.7623 | 0.5177 | 0.2474 | <0.0001 |
| Fc Control vs. TACI 13-118 – Fc | 0.7210 | 0.6151 | 0.2739 | 0.0001 |
| Fc Control vs. 406 BCMA -Fc | 0.8262 | 0.2192 | 0.4770 | <0.0001 |
| Fc Control vs. 381 BCMA -Fc | 0.2153 | 0.7029 | 0.0565 | <0.0001 |
| Fc Control vs. 411 BCMA -Fc | 0.2095 | 0.8683 | 0.0126 | <0.0001 |
| Fc Control vs. Naive | 0.0038 | 0.0086 | 0.0029 | <0.0001 |

| Table E26. Statistical Analysis of Splenic T Cell Subsets—Cell Numbers vs. Fc Control Group (FIG. 27) | | | | | | |
|---|---|---|---|---|---|---|
| **Comparison** | **Naïve CD4+ T cells** | **CD4+ Tcm cells** | **CD4+ Tem cells** | **Naïve CD8+ T cells** | **CD8+ Tcm cells** | **CD8+ Tem cells** |
| Fc Control vs. abatacept | 0.5081 | 0.0047 | <0.0001 | 0.6246 | 0.1115 | 0.3078 |
| Fc Control vs. 186 CTLA-4 vIgD-Fc | 0.9715 | 0.0017 | <0.0001 | 0.9728 | 0.3248 | 0.9740 |
| Fc Control vs. CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.0017 | <0.0001 | <0.0001 | 0.0109 | 0.0013 | 0.0391 |

| **Table E26. Statistical Analysis of Splenic T Cell Subsets—Cell Numbers vs. Fc Control Group (FIG. 27)** | | | | | | |
|---|---|---|---|---|---|---|
| **Comparison** | **Naïve CD4+ T cells** | **CD4+ Tcm cells** | **CD4+ Tem cells** | **Naïve CD8+ T cells** | **CD8+ Tcm cells** | **CD8+ Tem cells** |
| Fc Control vs. TACI 30-110 – Fc | 0.4484 | 0.0695 | 0.0088 | 0.9952 | 0.2531 | 0.1411 |
| Fc Control vs. TACI 13-118 – Fc | 0.4336 | 0.1831 | 0.0355 | 0.8153 | 0.3456 | 0.0729 |
| Fc Control vs. 406 BCMA -Fc | 0.7834 | 0.0995 | 0.0236 | 0.4410 | 0.0847 | 0.3897 |
| Fc Control vs. 381 BCMA -Fc | 0.2029 | 0.0003 | 0.0002 | 0.8184 | 0.6104 | 0.6897 |
| Fc Control vs. 411 BCMA -Fc | 0.0691 | 0.0002 | <0.0001 | 0.8360 | 0.6895 | 0.5270 |
| Fc Control vs. Naive | 0.0433 | 0.0516 | <0.0001 | 0.0782 | 0.0016 | 0.0166 |

| **Table E27. Statistical Comparisons Across All Treatment Groups for Numbers of T1 B Cells** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| abatacept | ns | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | ns | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.0417 | ns | 0.0164 | | | | | | |
| TACI 30-110 – Fc | ns | ns | ns | ns | | | | | |
| TACI 13-118 – Fc | ns | ns | ns | ns | ns | | | | |
| 406 BCMA - Fc | ns | ns | ns | ns | ns | ns | | | |
| 381 BCMA - Fc | ns | ns | ns | ns | ns | ns | ns | | |

| Table E27. Statistical Comparisons Across All Treatment Groups for Numbers of T1 B Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| 411 BCMA - Fc | ns | ns | ns | ns | ns | ns | ns | ns | |
| Naive | ns | ns | ns | ns | ns | ns | ns | ns | ns |

| Table E28. Statistical Comparisons Across All Treatment Groups for Numbers of T2 B cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA - Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| abatacept | ns | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | ns | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | <0.0001 | <0.0001 | | | | | | |
| TACI 30-110 – Fc | ns | 0.0142 | 0.0257 | <0.0001 | | | | | |
| TACI 13-118 – Fc | 0.0042 | <0.0001 | <0.0001 | <0.0001 | 0.0268 | | | | |
| 406 BCMA -Fc | <0.0001 | <0.0001 | <0.0001 | ns | <0.0001 | <0.0001 | | | |
| 381 BCMA -Fc | <0.0001 | <0.0001 | <0.0001 | ns | <0.0001 | <0.0001 | ns | | |
| 411 BCMA -Fc | <0.0001 | 0.0002 | 0.0002 | ns | <0.0001 | <0.0001 | ns | ns | |
| Naive | 0.0231 | ns | ns | <0.0001 | 0.0033 | <0.0001 | 0.0001 | <0.0001 | 0.0003 |

| Table E29. Statistical Comparisons Across All Treatment Groups for Numbers of Follicular B Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA - Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| abatacept | 0.0008 | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | 0.0185 | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | <0.0001 | <0.0001 | | | | | | |
| TACI 30-110 – Fc | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | |
| TACI 13-118 – Fc | <0.0001 | 0.0014 | 0.0001 | <0.0001 | ns | | | | |
| 406 BCMA - Fc | <0.0001 | <0.0001 | <0.0001 | ns | 0.0002 | <0.0001 | | | |
| 381 BCMA - Fc | <0.0001 | <0.0001 | <0.0001 | ns | <0.0001 | <0.0001 | ns | | |
| 411 BCMA - Fc | <0.0001 | <0.0001 | <0.0001 | ns | 0.0002 | <0.0001 | ns | ns | |
| Naive | <0.0001 | 0.0002 | <0.0001 | <0.0001 | ns | ns | <0.0001 | <0.0001 | <0.0001 |

| Table E30. Statistical Comparisons Across All Treatment Groups for Numbers of Marginal Zone B Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| abatacept | 0.0021 | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | 0.0003 | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | <0.0001 | <0.0001 | | | | | | |

**Table E30. Statistical Comparisons Across All Treatment Groups for Numbers of Marginal Zone B Cells**

| Treatment Group | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA -Fc | 411 BCMA - Fc |
|---|---|---|---|---|---|---|---|---|---|
| TACI 30-110 – Fc | <0.0001 | <0.0001 | <0.0001 | ns | | | | | |
| TACI 13-118 – Fc | <0.0001 | <0.0001 | <0.0001 | ns | ns | | | | |
| 406 BCMA - Fc | <0.0001 | <0.0001 | <0.0001 | ns | ns | ns | | | |
| 381 BCMA - Fc | <0.0001 | <0.0001 | <0.0001 | ns | ns | ns | ns | | |
| 411 BCMA - Fc | <0.0001 | <0.0001 | <0.0001 | ns | ns | ns | ns | ns | |
| Naive | <0.0001 | ns | ns | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

**Table E31. Statistical Comparisons Across All Treatment Groups for Numbers of Germinal Centre B Cells**

| Treatment Group | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA - Fc | 381 BCMA -Fc | 411 BCMA - Fc |
|---|---|---|---|---|---|---|---|---|---|
| abatacept | <0.0001 | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | <0.0001 | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | ns | ns | | | | | | |
| TACI 30-110 – Fc | 0.0182 | <0.0001 | 0.0002 | <0.0001 | | | | | |
| TACI 13-118 – Fc | 0.0139 | <0.0001 | 0.0002 | <0.0001 | ns | | | | |
| 406 BCMA - Fc | 0.0003 | 0.0058 | 0.0105 | 0.004 | ns | ns | | | |
| 381 BCMA - Fc | 0.0001 | 0.0113 | 0.0192 | 0.0079 | ns | ns | ns | | |

| Table E31. Statistical Comparisons Across All Treatment Groups for Numbers of Germinal Centre B Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA - Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| 411 BCMA - Fc | 0.0006 | 0.003 | 0.0058 | 0.002 | ns | ns | ns | ns | |
| Naive | <0.0001 | ns | ns | ns | <0.0001 | <0.0001 | 0.0063 | 0.0127 | 0.0032 |

| Table E32. Statistical Comparisons Across All Treatment Groups for Numbers of Plasma Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| abatacept | 0.0019 | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | ns | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | <0.0001 | <0.0001 | | | | | | |
| TACI 30-110 – Fc | <0.0001 | ns | 0.0392 | <0.0001 | | | | | |
| TACI 13-118 – Fc | 0.0016 | ns | ns | <0.0001 | ns | | | | |
| 406 BCMA - Fc | <0.0001 | 0.0237 | 0.0013 | 0.0052 | ns | 0.0268 | | | |
| 381 BCMA - Fc | <0.0001 | 0.0002 | <0.0001 | ns | 0.004 | 0.0003 | ns | | |
| 411 BCMA - Fc | <0.0001 | 0.0011 | <0.0001 | ns | 0.0151 | 0.0012 | ns | ns | |
| Naive | <0.0001 | 0.0184 | 0.0009 | 0.0036 | ns | 0.0211 | ns | ns | ns |

| Table E33. Statistical Comparisons Across All Treatment Groups for Numbers of CD3+ T Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| abatacept | ns | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | ns | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.0001 | 0.0113 | 0.0023 | | | | | | |
| TACI 30-110 – Fc | ns | ns | ns | 0.0004 | | | | | |
| TACI 13-118 – Fc | ns | ns | ns | 0.0004 | ns | | | | |
| 406 BCMA - Fc | ns | ns | ns | 0.0001 | ns | ns | | | |
| 381 BCMA - Fc | ns | ns | ns | 0.0059 | ns | ns | ns | | |
| 411 BCMA - Fc | ns | ns | ns | 0.0062 | ns | ns | ns | ns | |
| Naive | 0.0038 | ns | 0.0374 | ns | 0.0089 | 0.0104 | 0.0034 | ns | ns |

| Table E34. Statistical Comparisons Across All Treatment Groups for Numbers of CD4+ T Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| abatacept | ns | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | ns | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | 0.0049 | 0.0016 | | | | | | |
| TACI 30-110 – Fc | ns | ns | ns | 0.0008 | | | | | |
| TACI 13-118 – Fc | ns | ns | ns | 0.0007 | ns | | | | |

| Table E34. Statistical Comparisons Across All Treatment Groups for Numbers of CD4+ T Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| 406 BCMA - Fc | ns | ns | ns | 0.0005 | ns | ns | | | |
| 381 BCMA - Fc | ns | ns | ns | 0.0076 | ns | ns | ns | | |
| 411 BCMA - Fc | 0.0126 | ns | ns | 0.0367 | ns | ns | ns | ns | |
| Naive | 0.0029 | ns | ns | ns | 0.062 | ns | 0.033 | ns | ns |

| Table E35. Statistical Comparisons Across All Treatment Groups for Numbers of CD8+ T Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| abatacept | ns | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | ns | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.011 | ns | 0.0337 | | | | | | |
| TACI 30-110 – Fc | ns | ns | ns | 0.0024 | | | | | |
| TACI 13-118 – Fc | ns | ns | ns | 0.0034 | ns | | | | |
| 406 BCMA - Fc | ns | ns | ns | 0.0184 | ns | ns | | | |
| 381 BCMA - Fc | ns | ns | ns | 0.025 | ns | ns | ns | | |
| 411 BCMA - Fc | ns | ns | ns | 0.0076 | ns | ns | ns | ns | |
| Naive | 0.023 | ns | ns | ns | 0.0051 | 0.0072 | 0.0377 | 0.0507 | 0.0159 |

**Table E36. Statistical Comparisons Across All Treatment Groups for Numbers of Follicular Helper T Cells**

| Treatment Group | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA - Fc | 411 BCMA - Fc |
|---|---|---|---|---|---|---|---|---|---|
| abatacept | <0.0001 | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | <0.0001 | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | ns | ns | | | | | | |
| TACI 30-110 – Fc | <0.0001 | ns | 0.0242 | 0.0047 | | | | | |
| TACI 13-118 – Fc | 0.0001 | 0.0193 | 0.0026 | 0.0003 | ns | | | | |
| 406 BCMA - Fc | <0.0001 | ns | ns | 0.0314 | ns | ns | | | |
| 381 BCMA - Fc | <0.0001 | ns | ns | ns | ns | 0.0365 | ns | | |
| 411 BCMA - Fc | <0.0001 | ns | ns | ns | ns | 0.0485 | ns | ns | |
| Naive | <0.0001 | ns | 0.0159 | 0.0026 | ns | ns | ns | ns | ns |

**Table E37. Statistical Comparisons Across All Treatment Groups for Numbers of Naïve CD4+ T Cells**

| Treatment Group | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA - Fc | 381 BCMA - Fc | 411 BCMA -Fc |
|---|---|---|---|---|---|---|---|---|---|
| abatacept | ns | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | ns | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.0017 | 0.0108 | 0.0033 | | | | | | |
| TACI 30-110 – Fc | ns | ns | ns | 0.0139 | | | | | |

| Table E37. Statistical Comparisons Across All Treatment Groups for Numbers of Naïve CD4+ T Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA - Fc | 381 BCMA - Fc | 411 BCMA -Fc |
| TACI 13-118 – Fc | ns | ns | ns | 0.0148 | ns | | | | |
| 406 BCMA - Fc | ns | ns | ns | 0.0064 | ns | ns | | | |
| 381 BCMA - Fc | ns | ns | ns | 0.0484 | ns | ns | ns | | |
| 411 BCMA - Fc | ns | ns | ns | ns | ns | ns | ns | ns | |
| Naive | 0.0433 | ns | ns | ns | ns | ns | ns | ns | ns |

| Table E38. Statistical Comparisons Across All Treatment Groups for Numbers of CD4+ Tcm Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA - Fc | 381 BCMA - Fc | 411 BCMA -Fc |
| abatacept | 0.0047 | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | 0.0017 | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | 0.0019 | 0.0131 | | | | | | |
| TACI 30-110 – Fc | ns | ns | ns | <0.0001 | | | | | |
| TACI 13-118 – Fc | ns | ns | 0.0462 | <0.0001 | ns | | | | |
| 406 BCMA - Fc | ns | ns | ns | 0.0001 | ns | ns | | | |
| 381 BCMA - Fc | 0.0003 | ns | ns | 0.0212 | 0.0495 | 0.0151 | ns | | |
| 411 BCMA - Fc | 0.0002 | ns | ns | 0.0291 | 0.0368 | 0.0107 | 0.0414 | ns | |
| Naive | ns | ns | ns | <0.0001 | ns | ns | ns | 0.0461 | 0.0337 |

| Table E39. Statistical Comparisons Across All Treatment Groups for Numbers of CD4+ Tem Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA - Fc | 411 BCMA - Fc |
| abatacept | <0.0001 | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | <0.0001 | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | <0.0001 | 0.0003 | 0.0009 | | | | | | |
| TACI 30-110 – Fc | 0.0088 | ns | ns | <0.0001 | | | | | |
| TACI 13-118 – Fc | 0.0355 | 0.027 | 0.0288 | <0.0001 | ns | | | | |
| 406 BCMA - Fc | 0.0236 | ns | ns | <0.0001 | ns | ns | | | |
| 381 BCMA - Fc | 0.0002 | ns | ns | <0.0001 | ns | ns | ns | | |
| 411 BCMA - Fc | <0.0001 | ns | ns | 0.0012 | 0.0365 | 0.0091 | 0.0282 | ns | |
| Naive | <0.0001 | ns | ns | 0.0021 | 0.0128 | 0.0026 | 0.0103 | ns | ns |

| Table E40. Statistical Comparisons Across All Treatment Groups for Numbers of Naïve CD8+ T Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA - Fc | 411 BCMA - Fc |
| abatacept | ns | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | ns | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.0109 | 0.0362 | 0.0147 | | | | | | |
| TACI 30-110 – Fc | ns | ns | ns | 0.0107 | | | | | |

| Table E40. Statistical Comparisons Across All Treatment Groups for Numbers of Naïve CD8+ T Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA -Fc | 381 BCMA - Fc | 411 BCMA - Fc |
| TACI 13-118 – Fc | ns | ns | ns | 0.0197 | ns | | | | |
| 406 BCMA - Fc | ns | ns | ns | 0.0019 | ns | ns | | | |
| 381 BCMA - Fc | ns | ns | ns | 0.0195 | ns | ns | ns | | |
| 411 BCMA - Fc | ns | ns | ns | 0.0184 | ns | ns | ns | ns | |
| Naive | ns | ns | ns | ns | ns | ns | 0.016 | ns | ns |

| Table E41. Statistical Comparisons Across All Treatment Groups for Numbers of CD8+ Tcm Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA - Fc | 381 BCMA -Fc | 411 BCMA - Fc |
| abatacept | ns | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | ns | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.0013 | ns | 0.0303 | | | | | | |
| TACI 30-110 – Fc | ns | 0.0077 | 0.0422 | <0.0001 | | | | | |
| TACI 13-118 – Fc | ns | 0.0131 | ns | <0.0001 | ns | | | | |
| 406 BCMA - Fc | ns | 0.0019 | 0.0118 | <0.0001 | ns | ns | | | |
| 381 BCMA - Fc | ns | ns | ns | 0.0054 | ns | ns | 0.0294 | | |
| 411 BCMA - Fc | ns | 0.0485 | ns | 0.0004 | ns | ns | ns | ns | |
| Naive | 0.0016 | ns | 0.0408 | ns | <0.0001 | <0.0001 | <0.0001 | 0.0072 | 0.0004 |

| Table E42. Statistical Comparisons Across All Treatment Groups for Numbers of CD8+ Tem Cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment Group** | Fc control | abatacept | 186 CTLA-4 vIgD-Fc | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | TACI 30-110 – Fc | TACI 13-118 – Fc | 406 BCMA - Fc | 381 BCMA -Fc | 411 BCMA -Fc |
| abatacept | ns | | | | | | | | |
| 186 CTLA-4 vIgD-Fc | ns | ns | | | | | | | |
| CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.0391 | ns | 0.0476 | | | | | | |
| TACI 30-110 – Fc | ns | 0.0148 | ns | 0.0007 | | | | | |
| TACI 13-118 – Fc | ns | 0.0061 | ns | 0.0002 | ns | | | | |
| 406 BCMA - Fc | ns | ns | ns | 0.0061 | ns | ns | | | |
| 381 BCMA - Fc | ns | ns | ns | ns | ns | 0.03 | ns | | |
| 411 BCMA - Fc | ns | ns | ns | 0.0081 | ns | ns | ns | ns | |
| Naive | 0.0166 | ns | 0.0223 | ns | 0.0002 | <0.0001 | 0.0022 | 0.0447 | 0.0028 |

**[0747]** Together, these results indicate that BCMA vTD-containing single domain Fc fusion molecules, or the multi-stack domain molecule (CTLA-4 186 GSG4S Fc (G4S)4 TACI 541 (SEQ ID NO: 610)), that inhibit B and/or T cell activity can reduce immune responses and cell subset changes mediated by the T cell-dependent antigen KLH *in vivo* (i.e. anti-KLH levels in serum and changes in immune cell subsets). These results are consistent with the evaluation of the CTLA-4 and BCMA/TACI multidomain stack proteins, or single TACI or BCMA domain B cell inhibitory molecules, as clinical therapeutics in the treatment of autoimmune and inflammatory diseases in which hyperactive lymphocytes play a role.

**Example 14. Evaluation in Sjogren's syndrome model in non-obese diabetic mice**

**[0748]** This Example describes the assessment of exemplary single-domain 186-CTLA4 -Fc fusion protein (variant CTLA-4 SEQ ID NO: 186 fused to Fc set forth in SEQ ID NO:589), and multi-domain

stack molecule CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 (multidomain stack Fc fusion set forth in SEQ ID NO: 610) in an *in vivo* short term model of Sjogren's syndrome in NOD mice, including assessment of sialadenitis, serum levels of test molecules and insulitis.

**[0749]** The Sjogren's syndrome model was induced in female diabetes-prone NOD/ShiLtJ mice (about 6 weeks of age) by repeat dosing of an anti-mPD-L1 antibody. Specifically, 0.1 mg of anti-mPD-L1 antibody was administered by intraperitoneal injection on days 0, 2, 4, and 6 . Test molecule fusion proteins were dosed on days 0, 2 and 4 according to Table E43 below.

Table E43. Treatment Groups and Dosing Regimens

| Group | N | Anti-mPD-L1 Treatment (IP) | Test Article (TA) | TA Dose Level (IP) | mAb Treatment and TA dosing Days |
|---|---|---|---|---|---|
| 1 | 15 | 0.1 mg | Fc control | 0.28 mg | 0, 2, 4, 6 and 0,2 4 |
| 2 | 15 | 0.1 mg | 186-CTLA4 - Fc | 0.42 mg | 0, 2, 4, 6 and 0,2 4 |
| 4 | 15 | 0.1 mg | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 0.5 mg | 0, 2, 4, 6 and 0,2 4 |
| 6 (naïve) | 5 | 0 | n/a | 0 | n/a |

Abbreviations: IP= intraperitoneal(ly); mg= milligram; n/a = not applicable

**[0750]** Blood was obtained from the tail vein of mice (2-5 µL) on days 7, 8, 9, and 10, placed on a ReliOn Prime glucose test strip, and blood glucose (mg/dL) was measured using the ReliOn Prime Glucose Test System. At Day 10 of the experiment, mice were sacrificed and serum, submandibular glands (SMG), and pancreas were collected and analyzed.

**[0751]** The left SMG and pancreas were removed, dissected away from adjacent lymph nodes, and placed into neutral-buffered formalin (NBF) for approximately 72 hours, followed by transfer to 70% ethanol. The fixed tissues were embedded in paraffin, sectioned, and stained on glass slides with hematoxylin and eosin (H&E).

**[0752]** The scoring systems used to evaluate the extent of sialadenitis was scored as per Nandula et al. 2011 (Table 6 therein; reproduced as Table E44), and insulitis per Gutierrez et al 2014 (Table 7 therein; reproduced as Table E45).

| Table E44.Histological Scoring Used to Evaluate Sialadenitis | |
|---|---|
| Score | Criteria |
| 0 | No inflammatory foci |
| 1 | 1-5 foci of >50 inflammatory cells |
| 2 | >5 foci without parenchymal destruction |
| 3 | Moderate parenchymal destruction |
| 4 | Extensive parenchymal destruction |

| Table E45.Histological Scoring Used to Evaluate Insulitis | |
|---|---|
| Score | Criteria |
| 0 | No insulitis |
| 1 | Peri-islet insulitis |
| 2 | Intermediate insulitis |
| 3 | Intra-islet insulitis |
| 4 | Complete islet insulitis |

**[0753]** Statistically significant differences between groups for histology scores were determined using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test. Blood glucose levels were analyzed for significant differences using Kruskal-Wallis (non-parametric) test followed by Dunn's multiple comparison test. GraphPad PRISM® software (Version 8.1.2) was used for statistical analyses and $p$ values $< 0.05$ were considered statistically significant for all statistical tests.

**[0754]** Treatment with both exemplary CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 reduced incidence of Sialoadenitis (**FIG. 24A**) and resulted in a significantly lower histology score ($p<0.01$) than the mean scores for Fc control (**FIG. 24B**). These results are consistent with a finding that treatment of anti-PD-L1 injected NOD mice with the tested molecules reduced both the incidence and severity of sialadenitis in this model of Sjogren's syndrome.

**[0755]** The overall incidence of insulitis in these diabetes-prone mice and the degree of insulitis after treatment with the tested molecules is shown in **FIG. 25A** and **FIG. 25B**. CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 significantly reduced the degree of insulitis, as assessed by histological analysis (FIG. 25B).

**[0756]** **FIG. 26** depicts mean blood glucose concentrations (mg/dL) as measured in the blood on Days 7, 8, 9 and 10 for each tested group. As shown, blood glucose levels were significantly lower in the 186-CTLA-4 Fc and CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 treated groups compared to the Fc

control on Day 7 ($p<0.05$, $p<0.01$, and $p<0.001$, respectively). There was a trend for lower blood glucose levels with treatment with the multi-domain stack molecule CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 at other time points compared to the Fc control.

**[0757]** Together, these results indicate treatment with the tested exemplary multi-domain TACI-CTLA-4 molecule reduced the incidence and severity of sialadenitis in this mouse model of Sjogren's syndrome, and was generally effective at maintaining a lower blood glucose level over the course of study. These results indicate the potential for TACI-CTLA-4 containing multi-domain stack molecules in therapeutic use for treating Sjogren's syndrome, and for TACI-CTLA-4 multi-domain stack molecules as therapeutics to impact the onset of type 1 diabetes in humans.

**Example 15: Assessment of the Activity of Multi-Specific Constructs in an In Vivo Mouse Lupus Model.**

**[0758]** This Example describes the assessment of an exemplary CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 multi-domain molecule on immune responses in an *in vivo* murine bm12 inducible SLE model.

**[0759]** C57BL/6NJ (C57BL/6) mice were randomly assigned to the groups outlined in Table E45. Spleens from female I-$A^{bm12}$B6(c)-*H2*-$Ab1^{bm12}$/KhEgJ ('bm12')mice were processed aseptically to single cell suspensions in RPMI media and pooled into a total of 8mL. A total of 0.2mL of pooled bm12 splenocytes were adoptively transferred by injection via intraperitoneal delivery to 39 C57BL/6 "recipient" mice. Alloactivation of donor bm12 CD4+ T cells by recipient antigen presenting cells leads to chronic GVHD symptoms with symptoms closely resembling SLE, including autoantibody production, changes in immune cell subsets, and mild kidney disease. Glomerulonephritis with immune complex deposition develops late in the model, largely comprised of autoantigens bound to IgG1, IgG2b, IgG2c and IgG3 antibodies.

**[0760]** Recipient mice received doses of test molecules described in Table E45, starting at 1 hours after transfer of splenocytes, and then every 3-4 days, for a total of 22 doses; the last dose was administered 1 week prior to termination (last dose on Day 75). As a control, mice were treated with Fc control, WT full ECD TACI-Fc (abatacept) and TACI 30-110-Fc (TACI 30-110, SEQ ID NO:718; corresponding to the TACI ECD portion in atacicept). Five C57BL/6 and 5 bm12 mice were retained for use as naïve, untreated controls.

Table E46. Treatment Groups and Dosing Regimens

| Group | N | Splenocyte transfer on Day 0 | Test Articles | Dose (µg) | Dosing Regimen | Dosing Volume (IP) |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 10 | bm12→C57BL/6 | Fc control | 250 | Q3-4Dx22 | 0.1 mL |
| 2 | 9 | bm12→C57BL/6 | Abatacept | 400 | Q3-4Dx22 | 0.1 mL |
| 3 | 10 | bm12→C57BL/6 | TACI-30-110 Fc | 350 | Q3-4Dx22 | 0.1 mL |
| 4 | 10 | bm12→C57BL/6 | CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 | 460 | Q3-4Dx22 | 0.1 mL |
| 5 | 5 | None (naïve C57BL/6 mice) | Untreated (naïve C57BL/6 mice) | n/a | n/a | n/a |
| 6 | 5 | None (naïve bm12 mice) | Untreated (naïve bm12 mice) | n/a | n/a | n/a |
| Q3-4Dx22: every 3-4 days for a total of 22 doses. | | | | | | |

**[0761]** Statistically significant differences between groups for serum levels of anti-dsDNA antibodies, IgG isotypes, BUN, and CRE at specific time points were determined using one-way analysis of variance (ANOVA). Normality testing was performed to determine whether to use standard ANOVA (used for normally distributed data) or the non-parametric Kruskal-Wallis test. Multiple comparison tests between groups were performed using either uncorrected Fisher's Least Significant Difference (LSD) test (for standard ANOVA) or uncorrected Dunn's test (for Kruskal-Wallis). For flow cytometry data, significant differences between groups were analyzed using Student's unpaired, two-tailed T test. GraphPad PRISM® software (Version 8.1.2) was used for statistical analyses and p values $<0.05$ were considered statistically significant for all statistical tests.

*Blood Urea Nitrogen (BUN) and Serum Creatine (CRE) concentrations*

**[0762]** Serum collected at day 82 (study termination, 7 days after last dose) were analyzed for BUN and CRE. As shown in **FIG. 27**, the BUN concentrations were significantly elevated in the model as noted by the modestly higher concentrations in the Fc control group vs. the naïve C57BL/6 group ( $p<0.05$). Both the CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541- and the TACI 30-110-FC -Fc group each showed significantly lower BUN concentrations as compared to the abatacept-treated group. Overall, the increase in CRE levels in the Fc control group were minimal in this study and there were no significant differences between any of the groups.

*Serum IgG Isotype Concentration*

**[0763]** Serum was collected over time during the study and sample from Day 14, 42, and 82 were analyzed for concentrations of IgG2b, IgG2c, and IgG3 using Mouse IgG2b, Mouse IgG2c and Mouse IgG3 enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer's instructions. (Abcam; Cambridge, UK). Only day 82 (terminal) serum samples were analyzed for native bm12 mice. These IgG isotypes have previously been shown to be increased in immune complexes in the bm12 model, and thus are potentially pathogenic; specifically, the serum concentrations have been shown to increase during the first 4-5 weeks in the model and gradually decrease over time (Akieda et al., 2015 J. Immunol. 194(9):4162-74).

**[0764]** As shown in **FIGS. 28A-28C**, serum collected from Days 14, 42 and 82 show that treatment with the exemplary TACI-CTLA-4 multi-domain stack molecule CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 resulted in consistently and sustained low levels of each of the IgG isotypes throughout the study, being significantly different than both the Fc control and TACI 30-110-FC -Fc groups for each IgG isotype and at each of the time points tested. Although the control molecule WT TACI -Fc (abatacept) resulted in significantly lower levels of each IgG isotype as compared to the Fc only control at the Day 14 time point and in lower IgG2b concentrations at Day 82, the exemplary TACI-CTLA-4 multi-domain stack molecule resulted in significantly lower levels of each of the IgG isotypes as compared to abatacept at the Day 42 and Day 82 time points. Overall, treatment of mice with the exemplary TACI-CTLA-4 multi-domain stack molecule reduced the levels of these pathogenic IgG isotypes in serum as much as, or more so than, abatacept, and always more so than the TACI 30-110-FC -Fc.

*Serum Anti-dsDNA Antibody Analysis*

**[0765]** Serum was collected over time during the study and samples from Day 7, 14, 28, 42, 70, and 82 were analyzed for concentrations of anti-dsDNA antibodies using a Mouse Anti-dsDNA IgG Antibody Assay Kit according to the manufacturer's instructions (Chondrex, Inc; Redmond, WA). Similar to the serum IgG isotype patterns over time, anti-dsDNA antibody levels peaked at about 6 weeks and gradually diminished over the next several weeks. As shown in **FIG. 29** serum collected from subsets of mice in each treatment group on Days 7, 14, 28, 42, 70, and 82 and assayed by ELISA showed consistently low levels of anti-dsDNA antibody levels at each time point for the exemplary TACI-CTLA-4 multi-domain stack molecule, CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 treatment group. Treatment with WT TACI-Fc (abatacept) resulted in significantly lower concentrations of anti-dsDNA than the Fc control group at Day 14, 28 and 42, however, treatment with the exemplary TACI-CTLA-4 multi-domain stack molecule were significantly lower than in the abatacept group at Day 7 and Day 82. These results show that the exemplary TACI-CTLA-4 multi-domain stack molecule consistently reduced

concentrations of serum anti-dsDNA antibodies, an important pathogenic antibody in SLE, more potently than abatacept.

*Immunophenotyping of Lymphocyte Subsets*

**[0766]** Mice were sacrificed on Day 82, and blood and tissues were collected as follows: cervical lymph node (LN) and part of the spleen were placed in Dulbecco's phosphate buffered saline on ice for flow cytometry. This half of spleen and the LN were each mechanically processed into single cell suspensions, the red blood cells (RBC) in the spleen cells lysed with 1X RBC Lysis Buffer (BioLegend), and the cells counted. One million live cells from spleen and LN preparations were stained with flow cytometry reagents, and immunophenotyped to track lymphocyte subsets.

**[0767]** **Table E47** sets forth the spleen weight, number of spleen cells (spleen cell #) and percentages of different cell subsets in spleens of treated mice as determined by flow immunophenotyping. **Table E48** sets forth the percentages of different cell subsets in cervical LN of treated mice.

**[0768]** The results demonstrated multiple effects on immune cell subsets associated with SLE, including reductions in the percentage and number of germinal center (GC) B cells and CD4+ $T_{FH}$ cells, compared to Fc control-treated mice. Further, treatment with the TACI-CTLA4-Fc multi-domain stack molecule reduced B220+ cells in spleen and LN compared to other treatment groups, spared immature T1 cells, but significantly reduced more mature marginal zone (MZ), T2, follicular B cells, and antibody-producing plasma cells in the spleen. Without wishing to be bound by theory, these results indicate that the B cell compartment could repopulate from the unaffected T1 cells following washout of the test molecule.

**[0769]** The results also demonstrated that treatment with the TACI-CTLA-Fc multi-domain stack molecule reduced CD4+ and CD8+ Tem and increased CD4+ and CD8+ Tcm cell subsets in spleen and LN compared to Fc control treatment. Further, treatment with the TACI-CTLA-Fc multi-domain stack molecule also reduced the percentage of ICOS+ CD4+ and CD8+ cells in spleen and LN compared to each of the other treatment groups.

**TABLE E47 : Spleen Immunophenotyping: Statistical Comparison Between Treatment Groups**

| **Data Set** | **Abatacept vs. Fc Control** | TACI-CTLA-4 multi-domain stack molecule **vs. Fc Control** | TACI-CTLA-4 multi-domain stack molecule **vs. Abatacept** | TACI-CTLA-4 multi-domain stack molecule **vs. TACI 30-110-FC** |
|---|---|---|---|---|
| Spleen weight | 0.002 | <0.0001 | <0.0001 | 0.0002 |
| Spleen cell # | ns | 0.0002 | 0.0017 | 0.0009 |

| | | | | |
|---|---|---|---|---|
| % CD45+ of live cells spleen | ns | ns | ns | ns |
| CD45+ cell #/spleen | ns | 0.0078 | 0.0145 | 0.0312 |
| % B220+ cells of CD45+ cells | ns | <0.0001 | <0.0001 | <0.0001 |
| B220+ cell #/spleen | ns | <0.0001 | <0.0001 | 0.0011 |
| % CD3+ cells of CD45+ cells | ns | <0.0001 | <0.0001 | <0.0001 |
| CD3+ cell #/spleen | ns | 0.0042 | 0.0219 | ns |
| % GC B cells of B220+ cells | 0.0037 | 0.0056 | ns | 0.0182 |
| GC B cell #/spleen | 0.0129 | 0.0011 | 0.001 | 0.0062 |
| % Plasma cells of B220+ cells | 0.0375 | <0.0001 | 0.0013 | 0.0011 |
| Plasma cell #/spleen | 0.0152 | 0.0011 | 0.0005 | 0.0279 |
| % MZ B cells of CD19+ cells | ns | <0.0001 | 0.0002 | 0.0416 |
| MZ B cell #/spleen | ns | <0.0001 | <0.0001 | ns |
| % T1 B cells of CD19+ cells | ns | <0.0001 | <0.0001 | <0.0001 |
| T1 B cell #/spleen | ns | ns | ns | ns |
| % T2 B cells of CD19+ cells | 0.0161 | <0.0001 | <0.0001 | <0.0001 |
| T2 B cell #/spleen | ns | <0.0001 | <0.0001 | ns |
| % Foll B cells of CD19+ cells | 0.0161 | <0.0001 | <0.0001 | <0.0001 |
| Foll B cell #/spleen | ns | <0.0001 | <0.0001 | 0.0017 |
| % CD4+ T cells of CD45+ cells | ns | <0.0001 | <0.0001 | 0.023 |
| CD4+ T cell #/spleen | ns | 0.0217 | ns | ns |
| % CD8+ T cells of CD45+ cells | 0.0013 | <0.0001 | <0.0001 | <0.0001 |
| CD8+ T cell #/spleen | ns | 0.0009 | 0.0049 | ns |
| CD4+ T cell % hIgG+ cells | 0.0319 | <0.0001 | 0.0005 | <0.0001 |
| CD8+ T cell % hIgG+ cells | ns | 0.0154 | 0.0075 | ns |
| CD45+ $CD3^-$ % hIgG+ non-T cells | ns | <0.0001 | <0.0001 | <0.0001 |
| CD4+ T cell % ICOS+ cells | <0.0001 | <0.0001 | 0.0082 | <0.0001 |
| CD4+ T cell ICOS+ cell #/spleen | ns | ns | ns | 0.0018 |
| CD8+ T cell % ICOS+ cells | 0.0009 | <0.0001 | 0.0171 | <0.0001 |
| CD8+ T cell ICOS+ cell #/spleen | ns | ns | ns | 0.0102 |
| CD4+ T cell % CD28+ cells | <0.0001 | <0.0001 | 0.0494 | 0.0002 |
| CD4+T cell CD28+ cell #/spleen | ns | 0.0154 | ns | ns |
| CD8+ T cell % CD28+ cells | 0.0379 | 0.0202 | ns | <0.0001 |
| CD8+T cell CD28+ cell #/spleen | ns | 0.0005 | 0.0051 | 0.0131 |
| CD4+ % Treg cells | <0.0001 | <0.0001 | 0.0001 | 0.0002 |
| CD4+ Treg cell #/spleen | 0.01 | 0.0002 | ns | 0.0033 |
| CD4+ % $T_{FH}$ cells | 0.0002 | <0.0001 | <0.0001 | 0.0005 |
| CD4+ $T_{FH}$ cell #/spleen | 0.0044 | 0.0009 | 0.0019 | 0.0061 |
| CD4+ % Naïve T cells | ns | <0.0001 | 0.0001 | 0.0001 |
| CD4+ Naive T cell #/spleen | ns | 0.0004 | 0.0131 | ns |
| CD4+ % Tem cells | <0.0001 | <0.0001 | 0.0002 | <0.0001 |
| CD4+ Tem cell #/spleen | 0.0287 | 0.0063 | ns | <0.0001 |

| | | | | |
|---|---|---|---|---|
| CD4+ % Tcm cells | <0.0001 | <0.0001 | ns | <0.0001 |
| CD4+ Tcm cell #/spleen | 0.0066 | <0.0001 | ns | 0.0037 |
| CD8+ % Naïve T cells | ns | 0.002 | 0.0078 | 49 |
| CD8+ Naive T cell #/spleen | ns | <0.0001 | 0.0013 | 0.0129 |
| CD8+ % Tem cells | 0.0033 | 0.0002 | 0.0026 | 0.0002 |
| CD8+ Tem cell #/spleen | 0.0156 | 0.0207 | ns | 0.0195 |
| CD8+ % Tcm cells | 0.0001 | ns | ns | ns |
| CD8+ Tcm cell #/spleen | ns | 0.001 | 0.0447 | ns |

ns = not significant

**TABLE E48. Cervical Lymph Node (LN) Immunophenotyping: Statistical Comparisons Between Treatment Groups**

| Data Set | Abatacept vs. Fc Control | TaBLI vs. Fc Control | TaBLI vs. Abatacept | TaBLI vs. TACI 30-110-FC |
|---|---|---|---|---|
| % CD45+ of Live cells | ns | ns | ns | ns |
| % B220+ of CD45+ cells | ns | <0.0001 | <0.0001 | 0.0003 |
| % CD3+ of CD45+ cells | ns | <0.0001 | <0.0001 | <0.0001 |
| % GC B cells of B220+ cells | 0.0237 | ns | 0.0324 | ns |
| % Plasma cells of B220+ cells | ns | 0.0002 | 0.0057 | 0.0005 |
| % MZ B cells of CD19+ cells | 0.0244 | 0.0029 | <0.0001 | 0.0378 |
| % T1 B cells of CD19+ cells | ns | <0.0001 | <0.0001 | <0.0001 |
| % T2 B cells of CD19+ cells | ns | ns | ns | 0.0005 |
| % Fol B cells of CD19+ cells | ns | <0.0001 | <0.0001 | <0.0001 |
| % CD4+ of CD45+ cells | ns | <0.0001 | <0.0001 | 0.0053 |
| % CD8+ of CD45+ cells | ns | <0.0001 | <0.0001 | <0.0001 |
| CD4+ % Treg cells | 0.0002 | <0.0001 | 0.0049 | <0.0001 |
| CD4+ % Tfh cells | 0.0029 | 0.0007 | 0.0002 | 0.0003 |
| CD4+ % hIgG+ cells | ns | ns | ns | ns |
| CD4+ % CD28+ cells | 0.0013 | 0.0004 | ns | 0.0001 |
| CD4+ % ICOS+ cells | 0.0012 | <0.0001 | 0.0387 | <0.0001 |
| CD8+ % hIgG+ cells | ns | ns | ns | 0.0249 |
| CD8+ % CD28+ cells | 0.0303 | 0.0001 | ns | 0.0001 |
| CD8+ % ICOS+ cells | 0.0408 | <0.0001 | 0.0459 | <0.0001 |
| CD45+ $CD3^-$ % hIgG+ non-T cells | ns | <0.0001 | <0.0001 | 0.0218 |
| CD4+ % Naïve T cells | ns | ns | ns | 0.0002 |
| CD4+ % Tem T cells | 0.0415 | 0.0107 | ns | ns |
| CD4+ % Tcm T cells | 0.0007 | 0.0003 | ns | 0.0437 |
| CD8+ % Naïve T cells | 0.0483 | ns | ns | ns |
| CD8+ % Tem T cells | 0.0192 | 0.0245 | ns | ns |
| CD8+ % Tcm T cells | 0.0189 | ns | ns | ns |

ns = not significant

*Conclusion*

**[0770]** These results support that a multi-domain TACI-CTLA-4 stack molecule, such as the exemplary CTLA4 186 - GSG4S - Fc - (G4S)4 - TACI 541 multi-domain stack molecule, is capable of inhibiting the expansion of key B and T lymphocyte subsets and reducing the formation of autoantibodies and potentially pathogenic IgG in an induced mouse model of chronic GVHD and SLE, in most cases more effectively than either TACI 30-110-Fc or abatacept. These findings indicate that inhibiting both the BAFF/APRIL B cell and CD80/86-CD28 costimulatory T cell pathways simultaneously may result in significant disease amelioration and control of pathogenic lymphocytes in SLE. Consequently, the dual pathway inhibitor achieved by a multi-domain TACI-CTLA-4 multi-domain stack molecule may be an effective treatment of SLE in humans.

## Example 16. Assessment of Exemplary Monomeric and Tetrameric Constructs.

**[0771]** Additional BCMA Fc fusion proteins were generated containing one (monomeric) or four (tetrameric barbell and tetrameric tandem) BCMA domains using the WT BCMA set forth in SEQ ID NO:356 and the BCMA vTD set forth in SEQ ID NO:406 (H19L). The monomeric and tetrameric BCMA WT and BCMA vTD were formatted as BCMA WT and BCMA vTD-Fc fusion proteins with an Fc domain. The exemplary generated Fc fusion proteins were generated substantially as described in Example 4, and are described in **Tables E49A-E49C**.

**[0772]** Briefly, to generate recombinant monomeric immunomodulatory proteins as single chain Fc fusion proteins, the encoding DNA was generated to encode a protein as follows: WT BCMA or variant BCMA domain followed by a linker of 12 amino acids (GSGGGGSGGGGS; SEQ ID NO: 805) followed by a single chain Fc (scFc) set forth in SEQ ID NO:852 (composed of a human IgG1 effectorless Fc sequence containing the mutations L234A, L235E and G237A, by the Eu Index numbering system for immunoglobulin proteins (SEQ ID NO:589) followed by a $(GGGGS)_{13}$ linker (SEQ ID NO:806) followed by the second a human IgG1 effectorless Fc sequence containing the mutations L234A, L235E and G237A, by the Eu Index numbering system for immunoglobulin proteins). The generated molecules are summarized in **Table E49A.**

**TABLE E49A.** Exemplary Monomeric Immunomodulatory Proteins

| AA SEQ ID NO | NT SEQ ID NO | DESCRIPTION | BCMA SEQ ID NO | LINKER SEQ ID NO | FC SEQ ID NO |
|---|---|---|---|---|---|
| 807 | 814 | BCMA 356 - GS(G4S)2 814- scFc 852 | 356 | 805 | 821 |

| | | | | | |
|---|---|---|---|---|---|
| 810 | 817 | BCMA 406-GS(G4S)2 814 -scFc 852 | 406 | 805 | 821 |

**[0773]** To generate recombinant tetrameric immunomodulatory proteins as Fc fusion proteins, proteins were generated in different formats as follows:

**[0774]** In one format, the encoding DNA was generated to encode three different protein versions as follows: BCMA Fc (SEQ ID NO:813): BCMA vTD domain SEQ ID NO:406 followed by a linker of (G4S)3 SEQ ID NO:595; followed by a BCMA vTD domain SEQ ID NO: 406; followed by a linker of GSGGGGS SEQ ID NO: 590; followed by a human IgG1 effectorless Fc sequence containing the mutations L234A, L235E and G237A, by the Eu Index numbering system for immunoglobulin proteins (SEQ ID NO:589).

**[0775]** In one format, the encoding DNA was generated to encode three different protein versions as follows: WT BCMA (SEQ ID NO:809): WT BCMA domain SEQ ID NO:356 followed by a linker of GSGGGGS SEQ ID NO: 590; followed by a human IgG1 effectorless Fc sequence containing the mutations L234A, L235E and G237A, by the Eu Index numbering system for immunoglobulin proteins (SEQ ID NO:589) followed by a linker of (G4S)3 SEQ ID NO: 595 followed by WT BCMA domain SEQ ID NO:356.

**[0776]** In one format, the encoding DNA was generated to encode three different protein versions as follows: BCMA Fc SEQ ID NO:812): BCMA vTD set forth in SEQ ID NO:406 followed by a linker of GSGGGGS SEQ ID NO: 590; followed by a human IgG1 effectorless Fc sequence containing the mutations L234A, L235E and G237A, by the Eu Index numbering system for immunoglobulin proteins (SEQ ID NO:589) followed by a linker of (G4S)3 SEQ ID NO: 595 followed by BCMA vTD set forth in SEQ ID NO:406.

| **TABLE E49B.** Exemplary Tetrameric Immunomodulatory Proteins | | | | | | | |
|---|---|---|---|---|---|---|---|
| AA SEQ ID NO | NT SEQ ID NO | DESCRIPTION | 1ST BCMA | LINKER | 2ND BCMA | LINKER | Fc |
| 813 | 820 | BCMA 406--(G4S)3 (595) BCMA 406--GSG4S (590) Fc 589 | 406 | 595 | 406 | 590 | 589 |

| **TABLE E49C.** Exemplary Tetrameric Immunomodulatory Proteins | | | | | | | |
|---|---|---|---|---|---|---|---|
| AA SEQ ID NO | NT SEQ ID NO | DESCRIPTION | 1ST BCMA | LINKER | FC | LINKER | 2ND BCMA |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 809 | 816 | BCMA 356 GSG4S (590) Fc 589 (G4S)3 (595) BCMA 356 | 356 | 590 | 589 | 595 | 356 |
| 812 | 819 | BCMA 406-- GSG4S (590) Fc 589- (G4S)3(595) BCMA 406- | 406 | 590 | 589 | 595 | 406 |

**A. Bioactivity of Exemplary Multi-Domain Molecules**

**[0777]** In one experiment, exemplary molecules set forth in Tables E49A-E49C were assessed using the Jurkat /NF-κB/TACI reporter cells for blockade of APRIL- or BAFF-mediated signaling. **Table E50** provides the values for half maximal inhibitory concentration (IC50) for inhibition of APRIL- and BAFF- mediated TACI signaling. In some instances, the proteins tested were not compared to their parental of WT controls and appear as (-) in the Table below. The results in Table **E50** demonstrate that all generated formats block both APRIL and BAFF mediated signaling.

**TABLE E50.** Assessment of Exemplary Monomeric and Tetrameric Immunomodulatory Proteins

| SEQ ID NO | Format | APRIL $IC_{50}$ ( pM ) | BAFF $IC_{50}$ ( pM ) | BAFF 60-mer $IC_{50}$ ( pM ) |
|---|---|---|---|---|
| 807 | BCMA 356 - GS(G4S)2 814-Fc 589-$(GGGGS)_{13}$ 815-Fc 589 | - | - | - |
| 809 | BCMA 356 GSG4S (590) Fc 589 (G4S)3 (595) BCMA 356 | 3255 | 3073 | 84875 |
| 810 | BCMA 406-- GS(G4S)2 814-Fc 589-$(GGGGS)_{13}$ 815-Fc 589 | - | - | - |
| 812 | BCMA 406-- GSG4S (590) Fc 589- (G4S)3(595) BCMA 406- | 2717 | 583 | 2324 |
| 813 | BCMA 406-- (G4S)3 (595) BCMA 406-- GSG4S (590) Fc 589 | 775 | 638 | 630 |

## Example 17. Generation of Multi-Domain T and B Cell Inhibitory Immunomodulatory Proteins

**[0778]** Multi-domain immunomodulatory proteins were generated as homodimeric Fc fusions containing (1) a wild-type CTLA-4 extracellular domain as a T cell inhibitory molecule (TIM); and (2) a wild-type TACI extracellular domain portion or a variant thereof as a B cell inhibitory molecule (BIM)

that binds to a B cell stimulatory receptor, such as BCMA or APRIL, generally as described in Example 7A.

**[0779]** The TIM (wild-type CTLA-4) or BIM (wild-type TACI or variant) of the multi-domain immunomodulatory protein were variously linked to the N- or C-terminus of an Fc region via a peptide linker, such as a GSGGGGS (SEQ ID NO: 590) and $(GGGGS)_4$ (SEQ ID NO:600). To generate homodimeric Fc fusions, various exemplary IgG1 Fc regions were used, including the sequences set forth in SEQ ID NO:589, 822, 823 and 824.

Table **E51** below sets forth exemplary generated multi-domain homodimeric immunomodulatory Fc fusion proteins.

**Table E51: Multi-Domain Immunomodulatory Proteins (Homodimer)**

| Description | DNA SEQ ID NO | Protein SEQ ID NO | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
|---|---|---|---|---|---|---|
| (CTLA4 WT ECD 1) GSG4S Fc 589 (G4S)4 (TACI 528) | 796 | 759 | CTLA-4 ECD (1) | 68-110 WT (528) | GSG4S (590) (G4S)4 (600) | Fc (589) |
| (CTLA4 WT ECD 1) GSG4S Fc 853 (G4S)4 (TACI 528) | 797 | 786 | CTLA-4 ECD (1) | 68-110 WT (528) | GSG4S (590) (G4S)4 (600) | Fc (853) |
| (CTLA4 WT ECD 1) GSG4S Fc 854 (G4S)4 (TACI 528) | 798 | 787 | CTLA-4 ECD (1) | 68-110 WT (528) | GSG4S (590) (G4S)4 (600) | Fc (854) |
| (CTLA4 WT ECD 1) GSG4S Fc | 799 | 788 | CTLA-4 ECD (1) | Q75E, R84Q(68-110) | GSG4S (590) | Fc (589) |

| Table E51: Multi-Domain Immunomodulatory Proteins (Homodimer) | | | | | | |
|---|---|---|---|---|---|---|
| Description | DNA SEQ ID NO | Protein SEQ ID NO | CTLA-4 (SEQ ID NO) | TACI (SEQ ID NO) | Linker (SEQ ID NO) | Fc (SEQ ID NO) |
| 589 (G4S)4 (TACI 542) | | | | (542) | (G4S)4 (600) | |
| (CTLA4 WT ECD 1) GSG4S Fc 853 (G4S)4 (TACI 542) | 800 | 789 | CTLA-4 ECD (1) | Q75E, R84Q(68-110) (542) | GSG4S (590) (G4S)4 (600) | Fc (853) |
| (CTLA4 WT ECD 1) GSG4S Fc 854 (G4S)4 (TACI 542) | 801 | 790 | CTLA-4 ECD (1) | Q75E, R84Q(68-110) (542) | GSG4S (590) (G4S)4 (600) | Fc (854) |
| (CTLA4 WT ECD 1) GSG4S Fc 855 (G4S)4 (TACI 528) | 802 | 791 | CTLA-4 ECD (1) | 68-110 WT (528) | GSG4S (590) (G4S)4 (600) | Fc (855) |
| (CTLA4 WT ECD 1) GSG4S Fc 855 (G4S)4 (TACI 542) | 803 | 792 | CTLA-4 ECD (1) | Q75E, R84Q(68-110) (542) | GSG4S (590) (G4S)4 (600) | Fc (855) |

**[0780]** The present invention is not intended to be limited in scope to the particular disclosed embodiments, which are provided, for example, to illustrate various aspects of the invention. Various modifications to the compositions and methods described will become apparent from the description and

teachings herein. Such variations may be practiced without departing from the true scope and spirit of the disclosure and are intended to fall within the scope of the present disclosure.

CLAIMS

**WHAT IS CLAIMED:**

~~1. An immunomodulatory protein comprising:~~

~~(1) at least one T cell inhibitory molecule (TIM) that binds to (i) a T cell stimulatory receptor, or (ii) a ligand of a T cell stimulatory receptor; and/or that antagonizes activity of a T cell stimulatory receptor; and~~

~~(2) at least one B cell inhibitory molecule (BIM) that binds to a ligand of a B cell stimulatory receptor and/or antagonizes activity of a B cell stimulatory receptor.~~

~~2. The immunomodulatory protein of claim 1, wherein the TIM binds to a ligand of a T cell stimulatory receptor.~~

~~3. The immunomodulatory protein of claim 2, wherein:~~
~~the T cell stimulatory receptor is CD28; and/or~~
~~the ligand of the T cell stimulatory receptor is CD80 or CD86.~~

~~4. The immunomodulatory protein of any of claims 1-3, wherein the TIM is a CTLA-4 extracellular domain or a binding portion thereof that binds to CD80 or CD86.~~

~~5. The immunomodulatory protein of claim 4, wherein the CTLA-4 extracellular domain or binding portion thereof is (i) the sequence of amino acids set forth in SEQ ID NO:1 or SEQ ID NO:2, (ii) a variant CTLA-4 sequence of amino acids that has at least 85% sequence identity to SEQ ID NO:1 or SEQ ID NO:2; or (iii) a portion of (i) or (ii) comprising an IgV domain.~~

~~6. The immunomodulatory protein of claim 4 or claim 5, wherein the CTLA-4 extracellular domain or the binding portion thereof is set forth in SEQ ID NO:1.~~

~~7. The immunomodulatory protein of claim 4 or claim 5, wherein the CTLA-4 extracellular domain or the binding portion thereof is a variant CTLA-4 sequence of amino acids that has at least 85% sequence identity to SEQ ID NO:1 or a portion thereof comprising the IgV domain, wherein the variant CTLA-4 sequence comprises one or more amino acid substitutions in SEQ ID NO:1 or the portion thereof comprising the IgV domain.~~

8. The immunomodulatory protein of claim 7, wherein the variant CTLA-4 sequence comprises the amino acid substitution C122S.

9. The immunomodulatory protein of any of claims 1-5, 7 and 8, wherein the CTLA-4 extracellular domain or the binding portion thereof is set forth in SEQ ID NO: 668.

10. The immunomodulatory protein of any of claims 7-9, wherein the variant CTLA-4 binds to the ectodomain of CD80 and CD86, optionally wherein binding affinity to one or both of CD80 and CD86 is increased compared to the sequence set forth in SEQ ID NO:1 or the portion thereof comprising the IgV domain.

11. The immunomodulatory protein of any of claims 7-8 and 10, wherein the one or more amino acid substitutions comprise amino acid substitutions selected from L12F, R16H, G29W, T53S, M56T, N58S, L63P, L98Q, or Y105L, or combinations thereof.

12. The immunomodulatory protein of any of claims 7-8, 10 and 11, wherein the one or more amino acid substitutions comprise G29W, L98Q and Y105L.

13. The immunomodulatory protein of any of claims 7-8 and 10-12, wherein the one or more amino acid substitutions are G29W/N58S/L63P/Q82R/L98Q/Y105L, L12F/R16H/G29W/M56T/L98Q/Y105L, T53S/L63P/L98Q, or G29W/L98Q/Y105L.

14. The immunomodulatory protein of any of claims 1-8 and 10-13, wherein the CTLA-4 extracellular domain or the binding portion thereof is set forth in any one of SEQ ID NO:92, SEQ ID NO:112, SEQ ID NO:165 or SEQ ID NO:186 or a portion thereof comprising the IgV domain.

15. The immunomodulatory protein of any of claims 1-14, wherein:
the ligand of a B cell stimulatory receptor is APRIL or BAFF; and/or
the B cell stimulatory receptor is TACI, BCMA, or BAFF receptor.

16. The immunomodulatory protein of any of claims 1-15, wherein the BIM is a TACI extracellular domain or a binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

~~17. The immunomodulatory protein of claim 16, wherein the TACI extracellular domain or the binding portion thereof is an extracellular domain sequence set forth as (i) the sequence of amino acids set forth in SEQ ID NO:709, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:709; or (iii) a portion of (i) or (ii) comprising one or both of a CRD1 domain and CRD2 domain that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.~~

~~18. The immunomodulatory protein of claim 16 or claim 17, wherein the TACI extracellular domain or the binding portion thereof comprises the CRD1 domain and the CRD2 domain.~~

~~19. The immunomodulatory protein of any of claims 16-18, wherein the TACI extracellular domain or the binding portion thereof is a truncated wild-type TACI extracellular domain set forth in SEQ ID NO: 516.~~

~~20. The immunomodulatory protein of claim 16 or claim 17, wherein the TACI extracellular domain or the binding portion thereof is a truncated wild-type TACI extracellular domain, that contains the cysteine rich domain 2 (CRD2) but lacks the entirety of the cysteine rich domain 1 (CRD1).~~

~~21. The immunomodulatory protein of claim 16, claim 17 or claim 20, wherein the TACI extracellular domain or the binding portion thereof is a truncated wild-type TACI extracellular domain, that consists of a contiguous sequence contained within amino acid residues 67-118 that includes amino acid residues 71-104, with reference to positions set forth in SEQ ID NO:709.~~

~~22. The immunomodulatory protein of claim 20 or claim 21, wherein the truncated wild-type TACI extracellular domain or the binding portion thereof is 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 59, 50 or 51 amino acids in length.~~

~~23. The immunomodulatory protein of any of claims 16, 17 and 20-22, wherein the TACI extracellular domain or the binding portion thereof is set forth in SEQ ID NO:528.~~

~~24. The immunomodulatory protein of claim 16 or claim 17, wherein the TACI extracellular domain or the binding portion thereof is a variant TACI polypeptide that comprises one or more amino acid substitutions in the extracellular domain (ECD) of a reference TACI polypeptide or a specific binding fragment thereof at positions selected from among 40, 59, 60, 61, 74, 75, 76, 77, 78, 79, 82, 83, 84, 85, 86, 87, 88, 92, 95, 97, 98, 99, 101, 102 and 103, corresponding to numbering of positions set forth in SEQ ID NO:709.~~

~~25. The immunomodulatory protein of claim 24, wherein the reference TACI polypeptide is a truncated polypeptide consisting of the extracellular domain of TACI or a specific binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.~~

~~26. The immunomodulatory protein of claim 24 or claim 25 wherein the reference TACI polypeptide comprises the sequence of amino acids set forth in SEQ ID NO:709, or a portion of thereof comprising one or both of a CRD1 domain and CRD2 domain that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.~~

~~27. The immunomodulatory protein of any of claims 24-26, wherein the reference TACI polypeptide comprises the CRD1 domain and the CRD2 domain.~~

~~28. The immunomodulatory protein of any of claims 24-27, wherein the reference TACI polypeptide is set forth in SEQ ID NO:516.~~

~~29. The immunomodulatory protein of any of claims 24-26, wherein the reference TACI polypeptide is a truncated wild-type TACI extracellular domain that contains the cysteine rich domain 2 (CRD2) but lacks the entirety of the cysteine rich domain 1 (CRD1), wherein the variant TACI polypeptide comprises one or more amino acid substitutions in the truncated wild-type TACI extracellular domain.~~

~~30. The immunomodulatory protein of claim 29, wherein the truncated wild-type TACI extracellular domain consists of a contiguous sequence contained within amino acid residues 67-118 that includes amino acid residues 71-104, with reference to positions set forth in SEQ ID NO:122.~~

~~31. The immunomodulatory protein of claim 29 or claim 30, wherein the truncated wild-type TACI extracellular domain is 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 59, 50 or 51 amino acids in length.~~

~~32. The immunomodulatory protein of any of claims 24-26 and 29-31, wherein the reference TACI polypeptide consists essentially of the CRD2 domain.~~

~~33. The immunomodulatory protein of any of claims 24-26 and 29-31, wherein the reference TACI polypeptide comprises the sequence set forth in SEQ ID NO:528.~~

34. The immunomodulatory protein of any of claims 24-26 and 29-33, wherein the reference TACI polypeptide is set forth in SEQ ID NO:528.

35. The immunomodulatory protein of any of claims 24-34, wherein the variant TACI polypeptide comprises one or more amino acid substitutions at positions selected from among 74, 75, 76, 77, 78, 79, 82, 83, 84, 85, 86, 87, 88, 92, 95, 97, 98, 99, 101, 102 and 103, corresponding to numbering set forth in SEQ ID NO:709.

36. The immunomodulatory protein of claim 35, wherein the one or more amino acid substitutions are selected from E74V, Q75E, Q75R, G76S, K77E, F78Y, Y79F, L82H, L82P, L83S, R84G, R84L, R84Q, D85E, D85V, C86Y, I87L, I87M, S88N, I92V, Q95R, P97S, K98T, Q99E, A101D, Y102D, F103S, F103V, F103Y, or a conservative amino acid substitution thereof.

37. The immunomodulatory protein of claim 35 or claim 36, wherein the one or more amino acid substitutions comprise at least one of E74V, K77E, Y79F, L82H, L82P, R84G, R84L, R84Q, D85V, or C86Y.

38. The immunomodulatory protein of any of claims 35-37, wherein the one or more amino acid substitutions comprise an amino acid substitution selected from the group consisting of Q75E, K77E, F78Y, R84G, R84Q, A101D and Y102D, or any combination thereof.

39. The immunomodulatory protein of any of claims 35-38, wherein the one or more amino acid substitutions are D85E/K98T, I87L/K98T, L82P/I87L, G76S/P97S, K77E/R84L/F103Y, Y79F/Q99E, L83S/F103S, K77E/R84Q, K77E/A101D, K77E/F78Y/Y102D, Q75E/R84Q, Q75R/R84G/I92V, K77E/A101D/Y102D, R84Q/S88N/A101D, R84Q/F103V, K77E/Q95R/A101D or I87M/A101D.

40. The immunomodulatory protein of any of claims 35-39, wherein the one or more amino acid substitutiosn are K77E/F78Y/Y102D, Q75E/R84Q, or R84G .

41. The immunomodulatory protein of any of claims 35-40, wherein the variant TACI polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the reference TACI polypeptide.

42. The immunomodulatory protein of claim 41, wherein the increased binding affinity for BAFF or APRIL is independently increased more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold or 60-fold.

43. The immunomodulatory protein of any of claims 35-42, wherein:

the variant TACI polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 517-527, 536, 537, 682-701; or

the variant TACI polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 529-535, 538-550, 673-681 or760-772.

44. The immunomodulatory protein of any of claims 35-43, wherein the variant TACI polypeptide is set forth in any one of SEQ ID NO:535, SEQ ID NO: 541, SEQ ID NO:542, or SEQ ID NO:688.

45. The immunomodulatory protein of any of claims 1-15, wherein the BIM is a BCMA extracellular domain or a binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

46. The immunomodulatory protein of claim 45, wherein the BCMA extracellular domain or the binding portion thereof is an extracellular domain sequence set forth as (i) the sequence of amino acids set forth in SEQ ID NO:356, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:356; or (iii) a portion of (i) or (ii) comprising a CRD domain.

47. The immunomodulatory protein of claim 45 or claim 46, wherein the BCMA polypeptide or the binding portion thereof is set forth in SEQ ID NO:356.

48. The immunomodulatory protein of claim 45 or claim 46, wherein the BCMA extracellular domain or the binding portion thereof is a variant BCMA polypeptide comprising one or more amino acid substitutions in the extracellular domain (ECD) of a reference BCMA polypeptide at positions selected from among 9, 10, 11, 14, 16, 19, 20, 22, 25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 and 48, corresponding to numbering set forth in SEQ ID NO:710.

49. An immunomodulatory protein comprising a variant BCMA polypeptide, wherein the variant BCMA polypeptide comprises one or more amino acid substitutions in the extracellular domain (ECD) of a reference BCMA polypeptide at positions selected from among 9, 10, 11, 14, 16, 19, 20, 22,

~~25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 and 48, corresponding to numbering of positions set forth in SEQ ID NO:710.~~

~~50. An immunomodulatory protein comprising a variant BCMA-Fc fusion protein, wherein the variant BCMA-Fc fusion protein comprises a variant BCMA polypeptide, an Fc region, and a linker between the BCMA polypeptide and Fc region, wherein the variant BCMA polypeptide comprises one or more amino acid substitutions in the extracellular domain (ECD) of an reference BCMA polypeptide corresponding to positions selected from among 9, 10, 11, 14, 16, 19, 20, 22, 25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 and 48 with reference to positions set forth in SEQ ID NO:710.~~

~~51. The immunomodulatory protein of any of claims 48-50, wherein the reference BCMA polypeptide is the extracellular domain of BCMA or a binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.~~

~~52. The immunomodulatory protein of any of claims 48-51, wherein the reference BCMA lacks an N-terminal methionine.~~

~~53. The immunomodulatory protein of any of claims 48-52, wherein the reference BCMA polypeptide comprises the sequence of amino acids set forth in SEQ ID NO:356, or a portion thereof comprising a CRD domain .~~

~~54. The immunomodulatory protein of any of claims 48-53, wherein the reference BCMA polypeptide is set forth in SEQ ID NO:356.~~

~~55. The immunomodulatory protein of any of claims 48-54, wherein the one or more amino acid substitutions are selected from S9G, S9N, S9Y, Q10E, Q10P, N11D, N11S, F14Y, S16A, H19A, H19C, H19D, H19E, H19F, H19G, H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R, H19S, H19T, H19V, H19W, H19Y, A20T, I22L, I22V, Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y, R27H, R27L, S29P, S30G, S30Y, N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y, T32I, T32S, L35A, L35M, L35P, L35S, L35V, L35Y, T36A, T36G, T36N, T36M, T36S, T36V, R39L, R39Q, A43E, A43S, V45A, V45D, V45I, T46A, T46I, N47D, N47Y, S48G, or a conservative amino acid substitution thereof.~~

~~56. The immunomodulatory protein of any of claims 48-55, wherein the one or more amino acid substitution comprise at least one amino acid substitution selected from H19F, H19K, H19L, H19M, H19R or H19Y.~~

~~57. The immunomodulatory protein of any of claims 48-56, wherein the one or more amino acid substitutions are H19Y/S30G; H19Y/V45A; F14Y/H19Y; H19Y/V45D; H19Y/A43E; H19Y/T36A; H19Y/I22V; N11D/H19Y; H19Y/T36M; N11S/H19Y; H19Y/L35P/T46A; H19Y/N47D; S9D/H19Y; H19Y/S30G/V45D; H19Y/R39Q; H19Y/L35P; S9D/H19Y/R27H; Q10P/H19Y/Q25H; H19Y/R39L/N47D; N11D/H19Y/N47D; H19Y/T32S; N11S/H19Y/S29P; H19Y/R39Q/N47D; S16A/H19Y/R39Q; S9N/H19Y/N31K/T46I; H19Y/R27L/N31Y/T32S/T36A; N11S/H19Y/T46A; H19Y/T32I; S9G/H19Y/T36S/A43S; H19Y/S48G; S9N/H19Y/I22V/N31D; S9N/H19Y/Q25K/N31D; S9G/H19Y/T32S; H19Y/T36A/N47Y; H19Y/V45A/T46I; H19Y/Q25K/N31D; H19Y/Q25H/R39Q/V45D; H19Y/T32S/N47D; Q10E/H19Y/A20T/T36S; H19Y/T32S/V45I; H19F/Q25E/N31L/L35Y/T36S; H19F/Q25F/N31S/T36S; H19I/Q25F/N31S/T36V; H19F/Q25V/N31M/T36S; H19Y/Q25Y/N31L/L35Y/T36S; H19F/Q25I/N31M/L35A/T36S; H19I/Q25L/N31L/L35Y/T36S; H19F/Q25L/N31G/L35P/T36A; H19Y/I22L/N31G; H19F/I22V/Q25M/N31P/T36M; H19Y/N31L/L35Y/T36S; H19L/S30G/N31H/L35A; H19L/Q25S/N31V/L35S/T36V; H19L/Q25S/S30Y/N31G/L35M/T36V; H19F/Q25F/N31L/L35Y/T36S; H19F/Q25F/N31S/T36G; H19F/I22V/Q25S/N31V/L35S/T36V; H19F/Q25G/N31S/L35V/T36N; H19L/Q25H/N31D/L35S; or H19F/Q25F/N31S/L35Y/T36S.~~

~~58. The immunomodulatory protein of any of claims 48-57, wherein the one or more amino acid substitutions are or comprise H19F, H19L, H19K, H19M, H19R, H10Y, N11D/H19Y/N47D, H19Y/R39Q/N47D; S16A/H19Y/R39Q, S9G/H19Y/T32S; H19Y/T36A/N47Y; or Q10E/H19Y/A20T/T36S.~~

~~59. The immunomodulatory protein of any of claims 48-58, wherein the one or more amino acid substitutions are or comprise S16A/H19Y/R39Q.~~

~~60. The immunomodulatory protein of any of claims 48-59, wherein the variant BCMA polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the reference TACI polypeptide.~~

~~61. The immunomodulatory protein of claim 60, wherein the increased binding affinity for BAFF or APRIL is independently increased more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold or 60-fold.~~

~~62. The immunomodulatory protein of any of claims 48-61, wherein the variant BCMA polypeptide has up to 10 amino acid substitutions compared to the reference BCMA polypeptide.~~

~~63. The immunomodulatory protein of an of claims 48-61, wherein the variant BCMA polypeptide has up to 5 amino acid substitutions compared to the reference BCMA polypeptide.~~

~~64. The immunomodulatory protein of any of claims 48-63, wherein the variant BCMA polypeptide has at least 90% sequence identity to SEQ ID NO:356.~~

~~65. The immunomodulatory protein of any of claims 48-64, wherein the variant BCMA polypeptide has at least 95% sequence identity to SEQ ID NO:356.~~

~~66. The immunomodulatory protein of any of claims 48-65, wherein the variant BCMA polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 357-435.~~

~~67. The immunomodulatory protein of any of claims 48-66, wherein the variant BCMA polypeptide is set forth in any one of SEQ ID NOS: 357, 377, 380, 381, 390, 391, 396, 402, 405, 406, 407, or 411.~~

~~68. The immunomodulatory protein of any of claims 49 and 51-67, comprising a heterologous moiety that is linked to the variant BCMA polypeptide.~~

~~69. The immunomodulatory protein of claim 68, wherein the heterologous moiety is a half-life extending moiety, a multimerization domain, a targeting moiety that binds to a molecule on the surface of a cell, or a detectable label.~~

~~70. The immunomodulatory protein of claim 69, wherein the half-life extending moiety comprises a multimerization domain, albumin, an albumin-binding polypeptide, Pro/Ala/Ser (PAS), a C-terminal peptide (CTP) of the beta subunit of human chorionic gonadotropin, polyethylene glycol (PEG), long unstructured hydrophilic sequences of amino acids (XTEN), hydroxyethyl starch (HES), an albumin-binding small molecule, or a combination thereof.~~

~~71. The immunomodulatory protein of any of claims 49 and 51-67, that is a BCMA-Fc fusion protein, wherein the variant BCMA polypeptide is linked to an Fc region of an immunoglobulin, optionally via a linker.~~

72. The immunomodulatory protein of any of claims 50-71, wherein the linker comprises a peptide linker and the peptide linker is selected from GSGGS (SEQ ID NO: 592), GGGGS (G4S; SEQ ID NO: 593), GSGGGGS (SEQ ID NO: 590), GGGGSGGGGS (2xGGGGS; SEQ ID NO: 594), GGGGSGGGGSGGGGS (3xGGGGS; SEQ ID NO: 595), GGGGSGGGGSGGGGSGGGGS (4xGGGGS, SEQ ID NO:600), GGGGSGGGGSGGGGSGGGGSGGGGS (5XGGGGS, SEQ ID NO: 671), GGGGSSA (SEQ ID NO: 596) or combinations thereof.

73. The immunomodulatory protein of any of claims 1-48 and 51-67, wherein the immunomodulatory protein is a single polypeptide chain comprising the at least one TIM and the at least one BIM separated by a linker.

74. The immunomodulatory protein of claim 73, wherein the at least one TIM is amino-terminal to the at least one BIM in the polypeptide.

75. The immunomodulatory protein of claim 73, wherein the at least one TIM is carboxy-terminal to the at least one BIM in the polypeptide.

76. The immunomodulatory protein of any of claims 73-75, wherein the linker comprises a peptide linker and the peptide linker is selected from GSGGS (SEQ ID NO: 592), GGGGS (G4S; SEQ ID NO: 593), GSGGGGS (SEQ ID NO: 590), GGGGSGGGGS (2xGGGGS; SEQ ID NO: 594), GGGGSGGGGSGGGGS (3xGGGGS; SEQ ID NO: 595), GGGGSGGGGSGGGGSGGGGS (4xGGGGS, SEQ ID NO:600), GGGGSGGGGSGGGGSGGGGSGGGGS (5XGGGGS, SEQ ID NO: 671), GGGGSSA (SEQ ID NO: 596), SEQ ID NO: 711 (1xEAAAK), SEQ ID NO: 712 (2xEAAAK), SEQ ID NO: 713 (3xEAAAK), SEQ ID NO: 714 (4xEAAAK), SEQ ID NO: 715 (5xEAAAK), SEQ ID NO: 665 (6xEAAAK), or combinations thereof.

77. The immunomodulatory protein of any of claims 1-48, 51-67 and 73-76, wherein the immunomodulatory protein comprises the sequence of amino acids set forth in any of SEQ ID NOS: 618-623 or 703-708, or a sequence that exhibits at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto and retains activity.

78. The immunomodulatory protein of any of claims 1-48 and 51-67, wherein the at least one TIM or the at least one BIM is linked to a multimerization moiety that promotes dimerization, wherein the immunomodulatory protein is a dimer.

79. The immunomodulatory protein of claim 78, wherein the multimerization domain is an immunoglobulin Fc region.

80. The immunomodulatory protein of any one of claims 50-72 and 79, wherein the immunoglobulin Fc region is a homodimeric Fc region and the immunomodulatory protein is a homodimer comprising two identical copies of the same polypeptide.

81. The immunomodulatory protein of any one of claims 50-72, 79 and 80, wherein the immunoglobulin Fc region is an IgG2 Fc domain, optionally wherein the IgG2 Fc domain comprises the sequence of amino acids set forth in SEQ ID NO: 726 or 822 or a sequence of amino acids that exhibits at least 95% sequence identity to SEQ ID NO:726 or 822.

82. The immunomodulatory protein of any one of claims 50-72, 79 and 80, wherein the immunoglobulin Fc region is an IgG4 Fc domain comprising the amino acid substitution S228P, optionally wherein the Fc domain comprises the sequence of amino acids set forth in SEQ ID NO: 728 or 823, or a sequence of amino acids that exhibits at least 95% sequence identity to SEQ ID NO: 728 or 823.

83. The immunomodulatory protein of any of claims 50-72, 79 and 80, wherein the immunoglobulin Fc is an IgG1 Fc domain, or is a variant thereof that exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, optionally as compared to a wild-type IgG1 Fc domain.

84. The immunomodulatory protein of any of claims 50-72, 79, 80 and 83, wherein the immunoglobulin Fc comprises the amino acid sequence set forth in SEQ ID NO: 597.

85. The immunomodulatory protein of any of claims 50-72, 79, 80, and 83, wherein the immunoglobulin Fc is a variant IgG1 Fc domain comprising one or more amino acid substitutions selected from L234A, L234V, L235A, L235E, G237A, S267K, R292C, N297G, and V302C, by EU numbering.

86. The immunomodulatory protein of claim 85, wherein the immunoglobulin Fc region contains the amino acid substitutions L234A, L235E an G237A by EU numbering.

87. The immunomodulatory protein of any of claims 50-72, 79, 80, 83, 85 and 86, wherein the Fc is a variant Fc comprising the amino acid sequence set forth in SEQ ID NO:589 or SEQ ID NO: 824.

~~88. The immunomodulatory protein of any of claims 50-72, 80 and 83-87, wherein the BCMA-Fc fusion protein comprises the structure: BCMA polypeptide (BCMA)-Linker-Fc region.~~

~~89. The immunomodulatory protein of any of claims 50-72, 80 and 83-88, wherein the BCMA-Fc fusion protein is set forth in SEQ ID NO:629.~~

~~90. An immunomodulatory BCMA-Fc fusion protein that is a homodimer comprising two identical copies of the BCMA-Fc fusion protein set forth in SEQ ID NO: 629 linked by a covalent disulfide bond.~~

~~91. The immunomodulatory protein of any of claims 50-72, 80 and 83-87, wherein the BCMA-Fc fusion protein comprises the structure: (BCMA)-Linker-Fc region-Linker-(BCMA).~~

~~92. The immunomodulatory protein of any of claims 50-72, 80, 83-87 and 91, wherein the BCMA-Fc fusion protein is set forth in SEQ ID NO: 809 or SEQ ID NO: 812.~~

~~93. The immunomodulatory protein of any of claims 50-72, 80 and 83-87, wherein the BCMA-Fc fusion protein comprises the structure: (BCMA)-Linker-(BCMA)-Linker-Fc region.~~

~~94. The immunomodulatory protein of any of claims 50-72, 80, 83-87 and 93, wherein the BCMA-Fc fusion protein is set forth in SEQ ID NO: 813.~~

~~95. The immunomodulatory protein of any of claims 50-72, 80, and 83-94, wherein the Fc fusion protein neutralizes APRIL and BAFF.~~

~~96. The immunomodulatory protein of claim 95, wherein:~~
~~the IC50 for neutralizing APRIL is less than 100 pM, less than 50 pM, less than 40 pM, less than 30 pM, less than 20 pM, less than 10 pM, less than 5 pM or less than 1 pM, or is any value between any of the foregoing; and/or~~
~~the IC50 for neutralizing BAFF is less than 400 pM, less than 300 pM, less than 200 pM, less than 100 pM, less than 75 pM, less than 50 pM, less than 25 pm, or less than 10 pM, or is any value between any of the foregoing.~~

~~97. The immunomodulatory protein of any of claims 50-72, 80, and 83-96, wherein:~~

~~the Fc fusion protein blocks binding of APRIL, BAFF, or an APRIL/BAFF heterotrimer to BCMA or TACI; and/or~~

~~the Fc fusion protein reduces the levels of circulating APRIL, BAFF, or an APRIL/BAFF in the blood following administration to a subject.~~

~~98. The immunomodulatory protein of any one of claims 80-87, wherein each polypeptide of the homodimer comprises the at least one TIM and the at least one BIM and wherein the at least one TIM is amino-terminal to the at least one BIM in each polypeptide.~~

~~99. The immunomodulatory protein of any one of claims 80-87, wherein each polypeptide of the homodimer comprises the at least one TIM and the at least one BIM and wherein the at least one TIM is carboxy-terminal to the at least one BIM in each polypeptide.~~

~~100. The immunomodulatory protein of any of claims 1-44, 78-87, 98 and 99, wherein the immunomodulatory protein comprises the sequence of amino acids set forth in any of SEQ ID NOS: 610-617, 624-627, 637, 638, 643, 644, 648, 653-654, and 759-792 or a sequence that exhibits at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto and retains activity.~~

~~101. The immunomodulatory protein of any of claims 1-44, 78-87 and 98-100, wherein the TIM is a wild-type CTLA-4 extracellular domain or a binding portion thereof, and the BIM is a TACI extracellular domain or a binding portion thereof comprising amino acid substitutions K77E/F78Y/Y102D, Q75E/R84Q, or R84G, corresponding to positions set forth in SEQ ID NO:709, optionally wherein the TIM is set forth in SEQ ID NO:1 and the BIM is set forth in SEQ ID NO:535, 541, 542, or 688.~~

~~102. The immunomodulatory protein of any of claims 1-44, 78-87, and 98-101, wherein the immunomodulatory protein comprises the sequence set forth in SEQ ID NO:611, SEQ ID NO:788, SEQ ID NO: 789, SEQ ID NO: 790, or SEQ ID NO: 792.~~

~~103. An immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 611 linked by a covalent disulfide bond.~~

~~104. An immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 788 linked by a covalent disulfide bond.~~

~~105. An immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 789 linked by a covalent disulfide bond.~~

~~106. An immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 790 linked by a covalent disulfide bond.~~

~~107. An immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 792 linked by a covalent disulfide bond.~~

~~108. The immunomodulatory protein of any of claims 1-44, 78-87 and 98-100, wherein the TIM is a wild-type CTLA-4 extracellular domain or a binding portion thereof, and the BIM is a truncated TACI extracellular domain comprising the CRD2 domain, optionally wherein the TIM is set forth in SEQ ID NO:1 and the BIM is set forth in SEQ ID NO:528.~~

~~109. The immunomodulatory protein of any of claims 1-44, 78-87, 98-100 and 108, wherein the immunomodulatory protein comprises the sequence set forth in SEQ ID NO:759, SEQ ID NO: 786 SEQ ID NO: 787 or SEQ ID NO: 791.~~

~~110. An immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 759 linked by a covalent disulfide bond.~~

~~111. An immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 786 linked by a covalent disulfide bond.~~

~~112. An immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 787 linked by a covalent disulfide bond.~~

~~113. An immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 791 linked by a covalent disulfide bond.~~

~~114. The immunomodulatory protein of any of claims 1-44, 78-87 and 98-100, wherein the TIM is a CTLA-4 extracellular domain or a binding portion thereof, comprising amino acid substitutions G29W/L98Q/Y105L corresponding to positions set forth in SEQ ID NO:1, and the BIM is a TACI extracellular domain or a binding portion thereof comprising amino acid substitutions~~

~~K77E/F78Y/Y102D, Q75E/R84Q, or R84G, corresponding to positions set forth in SEQ ID NO:709, optionally wherein the TIM is set forth in SEQ ID NO:186 and the BIM is set forth in SEQ ID NO:535, 541, 542, or 688.~~

~~115. The immunomodulatory protein of any of claims 1-44, 78-87, 98-100 and 114, wherein the immunomodulatory protein comprises he sequence set forth in SEQ ID NO:610.~~

~~116. An immunomodulatory protein comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 610 linked by a covalent disulfide bond.~~

~~117. The immunomodulatory protein of any of claims 1-15, 45-48, 51-67, 78-87, 98 and 99, wherein the immunomodulatory protein comprises the sequence of amino acids set forth in any of SEQ ID NOS: 601-609, 631-636, 645-647, 649-652, 655-659, or a sequence that exhibits at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto and retains activity.~~

~~118. The immunomodulatory protein of any of claims 1-15, 45-48, 51-67, 78-87, 98, 99 and 117, wherein the TIM is a wild-type CTLA-4 extracellular domain or a binding portion thereof, and the BIM is a BCMA extracellular domain or a binding portion thereof comprising the amino acid substitution H19L corresponding to positions set forth in SEQ ID NO:710, optionally wherein the TIM is set forth in SEQ ID NO:1 and the BIM is set forth in SEQ ID NO:406, more optionally wherein the immunomodulatory protein comprises the sequence set forth in SEQ ID NO:602.~~

~~119. An immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 602 linked by a covalent disulfide bond.~~

~~120. The immunomodulatory protein of any of claims 1-15, 45-48, 51-67, 78-87, 98, 99 and 117, wherein the TIM is a CTLA-4 extracellular domain or a binding portion thereof comprising the amino acid substitutions G29W/L98Q/Y105L corresponding to positions set forth in SEQ ID NO:1, and the BIM is a BCMA extracellular domain or a binding portion thereof comprising the amino acid substitution H19L with reference to positions set forth in SEQ ID NO:710, optionally wherein the TIM is set forth in SEQ ID NO:186 and the BIM is set forth in SEQ ID NO:406, more optionally wherein the immunomodulatory protein comprises the sequence set forth in SEQ ID NO:601.~~

~~121. An immunomodulatory protein that is a homodimer comprising two identical copies of the Fc fusion protein set forth in SEQ ID NO: 601 linked by a covalent disulfide bond.~~

~~122. The immunomodulatory protein of claim 79, wherein the immunoglobulin Fc region is a heterodimeric Fc region and the immunomodulatory protein is a heterodimer comprising a first and second polypeptide, wherein the first polypeptide comprises one of the at least one BIM or the at least one TIM and the second polypeptide comprises the other of the at least one BIM and the at least one TIM.~~

~~123. The immunomodulatory protein of claim 122, wherein the heterodimeric Fc comprises one or more amino acid modifications in a wild-type Fc domain to effect heterodimer formation between the polypeptides, optionally wherein the wild-type Fc region is an IgG1 Fc region.~~

~~124. The immunomodulatory protein of claim 123, wherein the one more amino acid modifications are selected from a knob-into-hole modification and a charge mutation to reduce or prevent self-association due to charge repulsion.~~

~~125. The immunomodulatory protein of any of claims 122-124, wherein the heterodimeric Fc region further comprises one or more amino acid substitutions to reduced binding affinity to an Fc receptor and/or reduced effector function, optionally as compared to a wild-type IgG1 Fc domain.~~

~~126. The immunomodulatory protein of claim 125, wherein the one or more amino acid substitutions are selected from L234A, L234V, L235A, L235E, G237A, S267K, R292C, N297G, and V302C, by EU numbering.~~

~~127. The immunomodulatory protein of claim 125 or claim 126, wherein the immunoglobulin Fc region contains the amino acid substitutions L234A, L235E an G237A by EU numbering.~~

~~128. The immunomodulatory protein of any of claims 122-127, wherein the heterodimer comprises a first polypeptide comprising the sequence of amino acids set forth in SEQ ID NO: 662 or 663 and a second polypeptide comprising the sequence of amino acids set forth in SEQ ID NO:660.~~

~~129. The immunomodulatory protein of any of claims 1-128, wherein:~~

~~the immunomodulatory protein blocks binding of APRIL, BAFF, or an APRIL/BAFF heterotrimer to BCMA or TACI; and/or~~

~~the immunomodulatory protein reduces the levels of circulating APRIL, BAFF, or an APRIL/BAFF in the blood following administration to a subject.~~

~~130. The immunomodulatory protein of any of claims 1-129, wherein the immunomodulatory protein reduces or inhibits B cell maturation, differentiation and/or proliferation.~~

~~131. The immunomodulatory protein of any of claims 1-48, 51-87 and 98-130, wherein:~~
~~the immunomodulatory protein blocks binding of CD80 or CD86 to a costimulatory receptor, optionally wherein the costimulatory receptor is CD28; and/or~~
~~the immunomodulatory protein reduces or inhibits T cell costimulation.~~

~~132. A nucleic acid molecule(s) encoding the immunomodulatory protein of any of claims 1-131.~~

~~133. A vector, comprising the nucleic acid molecule of claim 132.~~

~~134. The vector of claim 133 that is an expression vector.~~

~~135. The vector of claim 133 or claim 134, wherein the vector is a mammalian expression vector or a viral vector.~~

~~136. A cell, comprising the nucleic acid of claim 132 or the vector of any of any of claims 133-135.~~

~~137. A method of producing an immunomodulatory protein, comprising introducing the nucleic acid molecule of claim 132 or vector of any of claims 133-135 into a host cell under conditions to express the protein in the cell.~~

~~138. The method of claim 137, further comprising isolating or purifying the immunomodulatory protein from the cell.~~

~~139. An immunomodulatory protein produced by the method of claim 137 or claim 138.~~

~~140. A pharmaceutical composition, comprising the immunomodulatory protein of any of claims 1-131 and 139.~~

~~141. The pharmaceutical composition of claim 140, comprising a pharmaceutically acceptable excipient.~~

~~142. An article of manufacture comprising the pharmaceutical composition of any of claims 140 or claim 141 in a vial or container.~~

~~143. A kit comprising the article of manufacture of claim 142, and instructions for use.~~

~~144. A method of reducing an immune response in a subject, comprising administering the immunomodulatory protein of any of claims 1-131 or the pharmaceutical composition of claim 140 or claim 141 to a subject in need thereof.~~

~~145. The method of claim 144, wherein a B cell immune response is reduced in the subject, whereby B cell maturation, differentiation and/or proliferation is reduced or inhibited.~~

~~146. The method of claim 144 or claim 145, wherein circulating levels of APRIL, BAFF or an APRIL/BAFF heterotrimer are reduced in the subject.~~

~~147. The method of any of claims 144-146, wherein a T cell immune response is reduced in the subject, whereby T cell costimulation is reduced or inhibited.~~

~~148. The method of any of claims 144-147, wherein reducing the immune response treats a disease, disorder or condition in the subject.~~

~~149. A method of reducing circulating levels of APRIL, BAFF or an APRIL/BAFF heterotrimer in a subject comprising administering the immunomodulatory protein of any of claims 1-131 or the pharmaceutical composition of claim 140 or claim 141 to the subject.~~

~~150. A method of treating a disease, disorder or condition in a subject, comprising administering the immunomodulatory protein of any of claims 1-131 or the pharmaceutical composition of claim 140 or claim 141 to a subject in need thereof.~~

~~151. The method of claim 148 or claim 150, wherein the disease, disorder or condition is an autoimmune disease, and inflammatory condition, a B cell cancer, an antibody- mediated pathology, a renal disease, a graft rejection, graft versus host disease, or a viral infection.~~

~~152. The method of claim 148, claim 150 or claim 151, wherein the disease, disorder or condition is selected from the group consisting of Systemic lupus erythematosus (SLE); Sjögren's syndrome, scleroderma, Multiple sclerosis, diabetes, polymyositis, primary biliary cirrhosis, IgA nephropathy, IgA vasculitis, optic neuritis, amyloidosis, antiphospholipid antibody syndrome (APS), autoimmune polyglandular syndrome type II (APS II), autoimmune thyroid disease (AITD), Graves' disease, autoimmune adrenalitis and pemphigus vulgaris.~~

~~153. The method of any of claims 148 and 150-152, wherein the disease, disorder or condition is a B cell cancer and the cancer is myeloma.~~

~~154. A pharmaceutical composition of claim 140 or claim 141 for use in reducing an immune response in a subject.~~

~~155. Use of an immunomodulatory protein of any of claims 1-131 or the pharmaceutical composition of claim 140 or claim 141 in the manufacture of a medicament for reducing an immune response in a subject.~~

~~156. The pharmaceutical composition for use of claim 154 or the use of claim 155, wherein the immune response is a B cell immune response, wherein reducing the immune response reduces or inhibits B cell maturation, differentiation and/or proliferation.~~

~~157. The pharmaceutical composition for use or the use of any of claims 154-156, wherein reducing the immune response reduces circulating levels of APRIL, BAFF or an APRIL/BAFF heterotrimer in the subject.~~

~~158. The pharmaceutical composition for use or the use of any of claims 154-157, wherein a T cell immune response is reduced in the subject, whereby T cell costimulation is reduced or inhibited.~~

~~159. The pharmaceutical composition for use or the use of any of claims 154-158, wherein reducing the immune response treats a disease, disorder or condition in the subject.~~

~~160. A pharmaceutical composition of claim 140 or claim 141 for use in treating a disease, disorder or condition in a subject.~~

~~161. Use of an immunomodulatory protein of any of claims 1-131 or the pharmaceutical composition of claim 140 or claim 141 in the manufacture of a medicament for treating a disease, disorder or condition in a subject.~~

~~162. The pharmaceutical composition for use of claim 160 or the use of claim 161, wherein the disease, disorder or condition is an autoimmune disease, an inflammatory condition, a B cell cancer, an antibody- mediated pathology, a renal disease, a graft rejection, graft versus host disease, or a viral infection.~~

~~163. The pharmaceutical composition for use or the use of any of claims 159-162, wherein the disease, disorder or condition is selected from the group consisting of Systemic lupus erythematosus (SLE); Sjögren's syndrome, scleroderma, Multiple sclerosis, diabetes, polymyositis, primary biliary cirrhosis, IgA nephropathy, IgA vasculitis, optic neuritis, amyloidosis, antiphospholipid antibody syndrome (APS), autoimmune polyglandular syndrome type II (APS-II), autoimmune thyroid disease (AITD), Graves' disease, autoimmune adrenalitis and pemphigus vulgaris.~~

~~164. The pharmaceutical composition for use or the use of any of claims 159-162, wherein the disease, disorder or condition is a B cell cancer and the cancer is myeloma.~~

1. An immunomodulatory protein comprising:

(1) at least one T cell inhibitory molecule (TIM) that binds to (i) a T cell stimulatory receptor, or (ii) a ligand of a T cell stimulatory receptor; and/or that antagonizes activity of a T cell stimulatory receptor; and

(2) at least one B cell inhibitory molecule (BIM) that binds to a ligand of a B cell stimulatory receptor and/or antagonizes activity of a B cell stimulatory receptor.

2. The immunomodulatory protein of claim 1, wherein the TIM binds to a ligand of a T cell stimulatory receptor, optionally wherein:

the T cell stimulatory receptor is CD28; and/or

the ligand of the T cell stimulatory receptor is CD80 or CD86.

3. The immunomodulatory protein of claim 1 or claim 2, wherein the TIM is a CTLA-4 extracellular domain or a binding portion thereof that binds to CD80 or CD86.

4. The immunomodulatory protein of claim 3, wherein the CTLA-4 extracellular domain or binding portion thereof is:

(a) (i) the sequence of amino acids set forth in SEQ ID NO:1 or SEQ ID NO:2, (ii) a variant CTLA-4 sequence of amino acids that has at least 85% sequence identity to SEQ ID NO:1 or SEQ ID NO:2; or (iii) a portion of (i) or (ii) comprising an IgV domain;

(b) set forth in SEQ ID NO:1; or

(c) a variant CTLA-4 sequence of amino acids that has at least 85% sequence identity to SEQ ID NO:1 or a portion thereof comprising the IgV domain, wherein the variant CTLA-4 sequence comprises one or more amino acid substitutions in SEQ ID NO:1 or the portion thereof comprising the IgV domain.

5. The immunomodulatory protein of claim 4, wherein the variant CTLA-4 is of (c) and wherein the variant CTLA-4 binds to the ectodomain of CD80 and CD86, optionally wherein binding affinity to one or both of CD80 and CD86 is increased compared to the sequence set forth in SEQ ID NO:1 or the portion thereof comprising the IgV domain.

6. The immunomodulatory protein of claim 4 or claim 5, wherein the one or more amino acid substitutions comprise amino acid substitutions selected from M87T, L12F, R16H, G29W, T53S, M56T, N58S, L63P, L98Q, or Y105L, or combinations thereof.

7. The immunomodulatory protein of any of claims 1-6, wherein:

(a) the ligand of a B cell stimulatory receptor is APRIL or BAFF;

(b) the B cell stimulatory receptor is TACI, BCMA, or BAFF-receptor;

(c) and/or the BIM is a TACI extracellular domain or a binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

8. The immunomodulatory protein of claim 7, wherein the TACI extracellular domain or the binding portion thereof is an extracellular domain sequence set forth as (i) the sequence of amino acids set forth in SEQ ID NO:709, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:709; or (iii) a portion of (i) or (ii) comprising one or both of a CRD1 domain and CRD2 domain that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

9. The immunomodulatory protein of claim 7 or claim 8, wherein the TACI extracellular domain or the binding portion thereof:

(a) comprises the CRD1 domain and the CRD2 domain;

(b) is a truncated wild-type TACI extracellular domain set forth in SEQ ID NO: 516;

(c) is a truncated wild-type TACI extracellular domain, that contains the cysteine rich domain 2 (CRD2) but lacks the entirety of the cysteine rich domain 1 (CRD1)wherein the truncated wild-type TACI extracellular domain or the binding portion thereof is 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 59, 50 or 51 amino acids in length;

(d) is a truncated wild-type TACI extracellular domain, that consists of a contiguous sequence contained within amino acid residues 67-118 that includes amino acid residues 71-104, with reference to positions set forth in SEQ ID NO:709; or

(e) is set forth in SEQ ID NO:528.

10. The immunomodulatory protein of claim 7 or claim 8, wherein the TACI extracellular domain or the binding portion thereof is a variant TACI polypeptide that comprises one or more amino acid substitutions in the extracellular domain (ECD) of a reference TACI polypeptide or a specific binding fragment thereof at positions selected from among 40, 59, 60, 61, 74, 75, 76, 77, 78, 79, 82, 83, 84, 85, 86, 87, 88, 92, 95, 97, 98, 99, 101, 102 and 103, corresponding to numbering of positions set forth in SEQ ID NO:709.

11. The immunomodulatory protein of claim 10, wherein the reference TACI polypeptide is a truncated wild-type TACI extracellular domain that contains the cysteine rich domain 2 (CRD2) but lacks the entirety of the cysteine rich domain 1 (CRD1), wherein the variant TACI polypeptide comprises one or more amino acid substitutions in the truncated wild-type TACI extracellular domain.

12. The immunomodulatory protein of claim 10 or claim 11, wherein the reference TACI polypeptide is set forth in SEQ ID NO:528.

13. The immunomodulatory protein of any of claims 10-12, wherein the variant TACI polypeptide comprises one or more amino acid substitutions at positions selected from among 74, 75, 76, 77, 78, 79, 82, 83, 84, 85, 86, 87, 88, 92, 95, 97, 98, 99, 101, 102 and 103, corresponding to numbering set forth in SEQ ID NO:709.

14. The immunomodulatory protein of claim 13, wherein the one or more amino acid substitutions are selected from E74V, Q75E, Q75R, G76S, K77E, F78Y, Y79F, L82H, L82P, L83S, R84G, R84L, R84Q, D85E, D85V, C86Y, I87L, I87M, S88N, I92V, Q95R, P97S, K98T, Q99E, A101D, Y102D, F103S, F103V, F103Y, or a conservative amino acid substitution thereof.

15. The immunomodulatory protein of claim 13 or claim 14, wherein the one or more amino acid substitutions are D85E/K98T, I87L/K98T, L82P/I87L, G76S/P97S, K77E/R84L/F103Y, Y79F/Q99E, L83S/F103S, K77E/R84Q, K77E/A101D, K77E/F78Y/Y102D, Q75E/R84Q, Q75R/R84G/I92V, K77E/A101D/Y102D, R84Q/S88N/A101D, R84Q/F103V, K77E/Q95R/A101D or I87M/A101D.

16. The immunomodulatory protein of any of claims 13-15, wherein the variant TACI polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the reference TACI polypeptide, optionally wherein the increased binding affinity for BAFF or APRIL is independently increased more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold or 60-fold.

17. The immunomodulatory protein of any of claims 13-16, wherein:

the variant TACI polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 517-527, 536, 537, 682-701; or

the variant TACI polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 529-535, 538-550, 673-681 or760-772; more optionally wherein

the variant TACI polypeptide is set forth in any one of SEQ ID NO: 542, SEQ ID NO:535, SEQ ID NO: 541, or SEQ ID NO:688.

18. The immunomodulatory protein of any of claims 1-17, wherein the BIM is a BCMA extracellular domain or a binding portion thereof that binds to APRIL, BAFF, or a BAFF/APRIL heterotrimer.

19. The immunomodulatory protein of claim 18, wherein the BCMA extracellular domain or the binding portion thereof is an extracellular domain sequence set forth as (i) the sequence of amino acids set forth in SEQ ID NO:356, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:356; or (iii) a portion of (i) or (ii) comprising a CRD domain.

20. The immunomodulatory protein of claim 18 or claim 19, wherein the BCMA extracellular domain or the binding portion thereof is a variant BCMA polypeptide comprising one or more amino acid substitutions in the extracellular domain (ECD) of a reference BCMA polypeptide at positions selected from among 9, 10, 11, 14, 16, 19, 20, 22, 25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 and 48, corresponding to numbering set forth in SEQ ID NO:710.

21. An immunomodulatory protein comprising a variant BCMA polypeptide, wherein the variant BCMA polypeptide comprises one or more amino acid substitutions in the extracellular domain (ECD) of a reference BCMA polypeptide at positions selected from among 9, 10, 11, 14, 16, 19, 20, 22, 25, 27, 29, 30, 31, 32, 35, 36, 39, 43, 45, 46, 47 and 48, corresponding to numbering of positions set forth in SEQ ID NO:710.

22. The immunomodulatory protein of claim 21 that is a variant BCMA-Fc fusion protein, comprising a variant BCMA polypeptide linked to an Fc region, optionally by a linker.

23. The immunomodulatory protein of any of claims 20-22, wherein the reference BCMA polypeptide comprises the sequence of amino acids set forth in SEQ ID NO:356, or a portion thereof comprising a CRD domain and/or is set forth in SEQ ID NO:356.

24. The immunomodulatory protein of any of claims 20-23, wherein the one or more amino acid substitutions are selected from S9G, S9N, S9Y, Q10E, Q10P, N11D, N11S, F14Y, S16A, H19A, H19C, H19D, H19E, H19F, H19G, H19I, H19K, H19L, H19M, H19N, H19P, H19Q, H19R, H19S, H19T, H19V, H19W, H19Y, A20T, I22L, I22V, Q25E, Q25F, Q25G, Q25H, Q25I, Q25K, Q25L, Q25M, Q25S, Q25V, Q25Y, R27H, R27L, S29P, S30G, S30Y, N31D, N31G, N31H, N31K, N31L, N31M, N31P, N31S, N31V, N31Y, T32I, T32S, L35A, L35M, L35P, L35S, L35V, L35Y, T36A, T36G, T36N, T36M, T36S, T36V, R39L, R39Q, A43E, A43S, V45A, V45D, V45I, T46A, T46I, N47D, N47Y, S48G, or a conservative amino acid substitution thereof.

25. The immunomodulatory protein of any of claims 20-24, wherein the one or more amino acid substitutions are H19Y/S30G; H19Y/V45A; F14Y/H19Y; H19Y/V45D; H19Y/A43E; H19Y/T36A; H19Y/I22V; N11D/H19Y; H19Y/T36M; N11S/H19Y; H19Y/L35P/T46A; H19Y/N47D; S9D/H19Y; H19Y/S30G/V45D; H19Y/R39Q; H19Y/L35P; S9D/H19Y/R27H; Q10P/H19Y/Q25H; H19Y/R39L/N47D; N11D/H19Y/N47D; H19Y/T32S; N11S/H19Y/S29P; H19Y/R39Q/N47D; S16A/H19Y/R39Q; S9N/H19Y/N31K/T46I; H19Y/R27L/N31Y/T32S/T36A; N11S/H19Y/T46A; H19Y/T32I; S9G/H19Y/T36S/A43S; H19Y/S48G; S9N/H19Y/I22V/N31D; S9N/H19Y/Q25K/N31D; S9G/H19Y/T32S; H19Y/T36A/N47Y; H19Y/V45A/T46I; H19Y/Q25K/N31D; H19Y/Q25H/R39Q/V45D; H19Y/T32S/N47D; Q10E/H19Y/A20T/T36S; H19Y/T32S/V45I; H19F/Q25E/N31L/L35Y/T36S; H19F/Q25F/N31S/T36S; H19I/Q25F/N31S/T36V; H19F/Q25V/N31M/T36S; H19Y/Q25Y/N31L/L35Y/T36S; H19F/Q25I/N31M/L35A/T36S; H19I/Q25L/N31L/L35Y/T36S; H19F/Q25L/N31G/L35P/T36A; H19Y/I22L/N31G; H19F/I22V/Q25M/N31P/T36M; H19Y/N31L/L35Y/T36S; H19L/S30G/N31H/L35A; H19L/Q25S/N31V/L35S/T36V; H19L/Q25S/S30Y/N31G/L35M/T36V; H19F/Q25F/N31L/L35Y/T36S;

H19F/Q25F/N31S/T36G; H19F/I22V/Q25S/N31V/L35S/T36V; H19F/Q25G/N31S/L35V/T36N; H19L/Q25H/N31D/L35S; or H19F/Q25F/N31S/L35Y/T36S.

26. The immunomodulatory protein of any of claims 20-25, wherein the variant BCMA polypeptide has increased binding affinity to one or both of APRIL and BAFF compared to the reference TACI polypeptide, optionally wherein the increased binding affinity for BAFF or APRIL is independently increased more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold or 60-fold.

27. The immunomodulatory protein of any of claims 20-26, wherein the variant BCMA polypeptide has at least 95% sequence identity to SEQ ID NO:356.

28. The immunomodulatory protein of any of claims 20-27, wherein the variant BCMA polypeptide comprises the sequence set forth in any one of SEQ ID NOS: 357-435.

29. The immunomodulatory protein of any of claims 1-20 and 23-28, wherein the at least one TIM or the at least one BIM is linked to a multimerization moiety that promotes dimerization, wherein the immunomodulatory protein is a dimer, optionally wherein the multimerization domain is an immunoglobulin Fc region.

30. The immunomodulatory protein of any of claims 22-28, wherein the Fc fusion protein (a) neutralizes APRIL and BAFF; (b) blocks binding of APRIL, BAFF, or an APRIL/BAFF heterotrimer to BCMA or TACI; or (c) the Fc fusion protein reduces the levels of circulating APRIL, BAFF, or an APRIL/BAFF in the blood following administration to a subject.

31. The immunomodulatory protein of any of claims 1-30, wherein:

(a) the immunomodulatory protein blocks binding of APRIL, BAFF, or an APRIL/BAFF heterotrimer to BCMA or TACI;

(b) the immunomodulatory protein reduces the levels of circulating APRIL, BAFF, or an APRIL/BAFF in the blood following administration to a subject;

(c) the immunomodulatory protein reduces or inhibits B cell maturation, differentiation and/or proliferation;

(d) the immunomodulatory protein blocks binding of CD80 or CD86 to a costimulatory receptor, optionally wherein the costimulatory receptor is CD28; and/or

(e) the immunomodulatory protein reduces or inhibits T cell costimulation.

32. A nucleic acid molecule(s) encoding the immunomodulatory protein of any of claims 1-31.

33. A vector, comprising the nucleic acid molecule of claim 32.

34. A cell, comprising the nucleic acid of claim 32 or the vector of claim 33.

35. A method of producing an immunomodulatory protein, comprising introducing the nucleic acid molecule of claim 32 or vector of any of claims claim 33 into a host cell under conditions to express the protein in the cell, optionally further comprising isolating or purifying the immunomodulatory protein from the cell.

36. An immunomodulatory protein produced by the method of claim 35.

37. A pharmaceutical composition, comprising the immunomodulatory protein of any of claims 1-32 and 36, optionally comprising a pharmaceutically acceptable excipient.

38. An article of manufacture comprising the pharmaceutical composition of claim 37 in a vial or container.

39. A kit comprising the article of manufacture of claim 38, and instructions for use.

40. A method of reducing an immune response in a subject, comprising administering the immunomodulatory protein of any of claims 1-31 or the pharmaceutical composition of claim 37 to a subject in need thereof.

41. The method of claim 40, wherein reducing the immune response treats a disease, disorder, or condition in the subject.

42. A method of reducing circulating levels of APRIL, BAFF, or an APRIL/BAFF heterotrimer in a subject comprising administering the immunomodulatory protein of any of claims 1-31 or the pharmaceutical composition of claim 37.

43. A method of treating a disease, disorder or condition in a subject, comprising administering the immunomodulatory protein of any of claims 1-31 or the pharmaceutical composition of claim 37 to a subject in need thereof.

44. The method of claim 41 or claim 43, wherein the disease, disorder or condition is an autoimmune disease, and inflammatory condition, a B cell cancer, an antibody- mediated pathology, a renal disease, a graft rejection, graft versus host disease, or a viral infection.

45. The method of any of claims 41, 43 or 44, wherein the disease, disorder or condition is (a) selected from the group consisting of Systemic lupus erythematosus (SLE); Sjögren's syndrome, scleroderma, Multiple sclerosis, diabetes, polymyositis, primary biliary cirrhosis, IgA nephropathy, IgA vasculitis, optic neuritis, amyloidosis, antiphospholipid antibody syndrome (APS), autoimmune polyglandular syndrome type II (APS II), autoimmune thyroid disease (AITD), Graves' disease, autoimmune adrenalitis and pemphigus vulgaris or (b) a B cell cancer and the cancer is myeloma.

46. A pharmaceutical composition of claim 37 for use in reducing an immune response in a subject.

47. Use of an immunomodulatory protein of any of claims 1-31 or the pharmaceutical composition of claim 37 in the manufacture of a medicament for reducing an immune response in a subject.

48. A pharmaceutical composition of claim 37 for use in treating a disease, disorder, or condition in a subject.

49 Use of an immunomodulatory protein of any of claims 1-31 or the pharmaceutical composition of claim 37 in the manufacture of a medicament for treating a disease, disorder, or condition in a subject.

50. The pharmaceutical composition for use of claim 48 or claim 49, wherein the disease, disorder or condition is an autoimmune disease, an inflammatory condition, a B cell cancer, an antibody-mediated pathology, a renal disease, a graft rejection, graft versus host disease, or a viral infection.

51. The pharmaceutical composition for use or the use of any of claims 48-50, wherein the disease, disorder or condition is (a) selected from the group consisting of Systemic lupus erythematosus

(SLE); Sjögren's syndrome, scleroderma, Multiple sclerosis, diabetes, polymyositis, primary biliary cirrhosis, IgA nephropathy, IgA vasculitis, optic neuritis, amyloidosis, antiphospholipid antibody syndrome (APS), autoimmune polyglandular syndrome type II (APS II), autoimmune thyroid disease (AITD), Graves' disease, autoimmune adrenalitis and pemphigus vulgaris; or (b) a B cell cancer and the cancer is myeloma.