Although therapeutics are known that can modulate the IS, improved therapeutics are needed. Provided are immunomodulatory proteins, including soluble proteins or transmembrane immunomodulatory proteins capable of being expressed on cells, that meet such needs.

SUMMARY

[0005] In some examples, provided herein is a variant CD80 polypeptide containing an IgV domain or a specific binding fragment thereof, an IgC domain or a specific binding fragment thereof, or both, wherein the variant CD80 polypeptide contains one or more amino acid modifications, at one or more positions, in an unmodified CD80 or specific binding fragment thereof, corresponding to position(s) 7, 23, 26, 30, 34, 35, 46, 51, 55, 57, 58, 65, 71, 73, 78, 79, 82, and/or 84 with reference to numbering of SEQ ID NO: 2. In some examples, the one or more amino acid modifications in the unmodified CD80 or specific binding fragment thereof, correspond(s) to position(s) 26, 35, 46, 57, and/or 71 with reference to numbering of SEQ ID NO: 2. In any of the examples, the amino acid modification is an amino acid substitution, insertion or deletion.

[0005a] In one aspect, the present disclosure refers to a purified CD80-Fc fusion protein comprising one polypeptide operably linked to an Fc domain;

wherein the one polypeptide is a variant CD80 polypeptide comprising an IgV domain and wherein the IgV domain is the only CD80 portion of the variant CD80 polypeptide;

wherein the variant CD80 polypeptide comprises one or more amino acid modifications, wherein the one or more amino acid modifications comprise the amino acid modification E35D corresponding to position 35 with reference to numbering of positions set forth in SEQ ID NO:2 ~~at one or more positions in an unmodified CD80 corresponding to positions 35, 46, 71, 7, 23, 26, 30, 34, 51, 55, 57, 58, 65, 73, 78, 79, 82, or 84 with reference to numbering of positions set forth in SEQ ID NO:2~~;

wherein the variant CD80 polypeptide has at least 93% sequence identity to (i) the sequence of amino acids set forth in SEQ ID NO:2 or (ii) a portion of the sequence of amino acids of SEQ ID NO:2 comprising an IgV domain of CD80; and

wherein the variant CD80 polypeptide exhibits increased binding affinity to the ectodomain of human PD-L1 compared to the binding affinity of ~~the~~ an unmodified human CD80 for the ectodomain of human PD-L1.

[0005b] In one aspect, the present disclosure refers to a purified CD80-Fc fusion protein comprising the formula CD80-linker-Fc, wherein the CD80 is a variant CD80 polypeptide comprising the sequence of amino acids set forth in SEQ ID NO:199.

[0005c] In one aspect, the present disclosure refers to a purified CD80-Fc fusion protein comprising the formula CD80-linker-Fc, wherein the CD80 is a variant CD80 polypeptide comprising the sequence of amino acids set forth in SEQ ID NO:208.

[0070] In some examples, provided herein is a method of modulating an immune response in a subject, such as increasing or decreasing an immune response, containing administering a pharmaceutical composition provided herein to the subject. In one ~~aspect~~example, the present disclosure refers to use of the pharmaceutical composition disclosed herein in the manufacture of a medicament for modulating an immune response in a subject, optionally wherein the immune response is increased.

[0071] In some examples, provided herein is a method of modulating an immune response in a subject that includes administering an immunomodulatory protein provided herein, such as an immunomodulatory protein that exhibits increased binding affinity to PD-L1. In some examples, the immune response is increased. In some examples, the immunomodulatory protein is an immunomodulatory protein provided herein that exhibits Fc-dependent CD28 costimulation. In some examples, the immunomodulatory protein is an immunomodulatory protein provided herein that exhibits PD-L1-dependent CD28 costimulation, optionally wherein the immunomodulatory protein contains a variant CD80 polypeptide provided herein.

[0072] In some examples, provided herein is a method of modulating an immune response in a subject, containing administering the engineered cells provided herein. In some examples, the engineered cells are autologous to the subject. In some examples, the engineered cells are allogenic to the subject.

[0073] Also provided herein is a method of modulating an immune response in a subject, e.g., increasing or decreasing an immune response, including administering to the subject a variant CD80 polypeptide, or an immunomodulatory protein or conjugate containing the variant CD80 polypeptide or an engineered cell or infectious agent secreting or expressing the variant CD80 polypeptide, wherein the variant CD80 polypeptide binds to CTLA-4 with increased affinity or selectively compared to binding of the unmodified CD80 to the CTLA-4. In some examples, the variant CTLA-4 polypeptide contains one or more modifications as described herein.

[0074] In some examples of the method, the variant CTLA-4 polypeptide contains one or more amino acid modifications at one or more positions in an unmodified CD80 polypeptide or a specific binding fragment thereof corresponding to positions selected from 12, 13, 16, 18, 20, 22, 23, 24, 25, 26, 27, 30, 31, 33, 34, 35, 36, 38, 41, 42, 43, 44, 46, 47, 48, 51, 54, 55, 57, 58, 61, 62, 65, 67, 68, 69, 70, 71, 72, 73, 76, 77, 78, 79, 81, 82, 83, 84, 85, 86, 88, 90, 91, 92, 93, 94, and/or 95 with reference to positions set forth in SEQ ID NO:2. In some examples, the variant CD80 polypeptide contains one or more amino acid modifications at one or more positions in an unmodified CD80 or specific binding fragment thereof, corresponding to position(s) 23, 26, 30,

WHAT IS CLAIMED:

1. A purified CD80-Fc fusion protein comprising one polypeptide operably linked to an Fc domain;

wherein the one polypeptide is a variant CD80 polypeptide comprising an IgV domain and wherein the IgV domain is the only CD80 portion of the variant CD80 polypeptide;

wherein the variant CD80 polypeptide comprises one or more amino acid modifications, wherein the one or more amino acid modifications comprise the amino acid modification E35D corresponding to position 35 with reference to numbering of positions set forth in SEQ ID NO:2 ~~at one or more positions in an unmodified CD80 corresponding to positions 35, 46, 71, 7, 23, 26, 30, 34, 51, 55, 57, 58, 65, 73, 78, 79, 82, or 84 with reference to numbering of positions set forth in SEQ ID NO:2~~;

wherein the variant CD80 polypeptide has at least 93% sequence identity to (i) the sequence of amino acids set forth in SEQ ID NO:2 or (ii) a portion of the sequence of amino acids of SEQ ID NO:2 comprising an IgV domain of CD80; and

wherein the variant CD80 polypeptide exhibits increased binding affinity to the ectodomain of human PD-L1 compared to the binding affinity of ~~the~~ an unmodified human CD80 for the ectodomain of human PD-L1.

2. The purified CD80-Fc fusion protein of claim 1, wherein the one or more amino acid modifications further comprise one or more amino acid modifications~~in an unmodified CD80 or specific binding fragment thereof are~~ selected from E7D, T13A, T13R, S15P, S15T, C16R, H18A, H18C, H18F, H18I, H18T, H18V, V20A, V20I, V22D, V22I, V22L, E23D, E23G, E24D, L25S, A26D, A26E, A26G, A26H, A26K, A26N, A26P, A26Q, A26R, A26S, A26T, Q27H, Q27L, T28Y, I30F, I30T, I30V, Y31C, Y31S, Q33E, Q33K, Q33L, Q33R, K34E, ~~E35D, E35G,~~ K36R, T41S, M42I, M42V, M43L, M43T, D46E, D46N, D46V, M47F, M47I, M47L, M47V, M47Y, N48H, N48K, N48R, N48S, N48T, N48Y, P51A, Y53F, Y53H, K54E, K54N, K54R, N55D, N55I, T57A, T57I, I58V, I61F, I61V, T62A, T62N, N63A, N63D, L65P, I67L, I67V, V68E, V68I, V68L, I69F, L70M, A71D, A71G, L72V, R73H, R73S, P74S, D76H, E77A, G78A, T79A, T79I, T79L, T79M, T79P, E81G, E81K, C82R, V84A, V84I, L85E, L85M, L85Q,

K86M, Y87C, Y87D, Y87H, Y87Q, E88V, D90P, F92S, F92V, K93T, R94Q, R94W, E95D, E95V, L97M, and L97Q with reference to numbering of positions set forth in SEQ ID NO: 2.

3. The purified CD80-Fc fusion protein of claim 1 or claim 2, wherein: the variant CD80 polypeptide comprises amino acid modifications H18Y/E35D, E35D/D46E, E35D/D46V, E35D/M47I, E35D/M47L, E35D/M47V, E35D/V68M, E35D/L85M, E35D/L85Q; and/or the variant CD80 polypeptide comprises amino acid modifications E35D/M47L/V68M or E35D/M47V/V68M.

4. The purified CD80-Fc fusion protein of any one of claims 1-3, wherein the variant CD80 polypeptide comprises up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid modifications compared to the sequence of amino acids 35-135, 35-138, 37-138, or 35-141 of SEQ ID NO:1~~binding affinity of the variant CD80 polypeptide for the ectodomain of human PD-L1 is increased compared to binding affinity of the unmodified CD80 for the ectodomain of PD-L1~~.

5. The purified CD80-Fc fusion protein of any one of claims 1-4~~claim 4~~, wherein the increased 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, 50-fold, 60-fold, 80-fold, 100-fold, 150-fold, 200-fold, 250-fold, 300-fold, 400-fold, or 450-fold.

6. The purified CD80-Fc fusion protein of any of claims 1-5, wherein the variant CD80 polypeptide comprises amino acid modifications E35D/A71D, E35D/M47I, E35D/T57I/L70Q/A71D, E35D/A71D, E35D/I67L/A71D, E35D/M47I/L70M, E35D/A71D/L72V, E35D/M43L/L70M, A26P/E35D/M43I/L85Q/E88D, E35D/D46V/L85Q, H18Y/A26T/E35D/A71D/L85Q, E35D/M47L, E35D/M43I/A71D, E23G/A26S/E35D/T62N/A71D/L72V/L85M, V22L/E35D/M43L/A71G/D76H, A26E/E35D/M47L/L85Q, D46E/A71D, Y31H/E35D/T41S/V68L/K93R/R94W, A26E/Q33R/E35D/M47L/L85Q/K86E, A26E/Q33R/E35D/M47L/L85Q, E35D/M47L/L85Q, A26E/Q33L/E35D/M47L/L85Q, A26E/Q33L/E35D/M47L, H18Y/A26E/Q33L/E35D/M47L/L85Q, Q33L/E35D/M47I, H18Y/Q33L/E35D/M47I,

Q33L/E35D/D46E/M47I, Q33R/E35D/D46E/M47I, H18Y/E35D/M47L, Q33L/E35D/M47V, Q33L/E35D/M47V/T79A, Q33L/E35D/T41S/M47V, Q33L/E35D/M47I/L85Q, Q33L/E35D/M47I/T62N/L85Q, Q33L/E35D/M47V/L85Q, A26E/E35D/M43T/M47L/L85Q/R94Q, Q33R/E35D/K37E/M47V/L85Q, V22A/E23D/Q33L/E35D/M47V, E24D/Q33L/E35D/M47V/K54R/L85Q, S15P/Q33L/E35D/M47L/L85Q, E7D/E35D/M47I/L97Q, Q33L/E35D/T41S/M43I, E35D/M47I/K54R/L85E, Q33K/E35D/D46V/L85Q, Y31S/E35D/M47L/T79L/E88G, H18L/V22A/E35D/M47L/N48T/L85Q, Q27H/E35D/M47L/L85Q/R94Q/E95K, Q33K/E35D/M47V/K89E/K93R, E35D/M47I/E77A/L85Q/R94W, A26E/E35D/M43I/M47L/L85Q/K86E/R94W, Q27H/Q33L/E35D/M47V/N55D/L85Q/K89N, H18Y/V20A/Q33L/E35D/M47V/Y53F, Q33L/E35D/M47L/A71G/F92S, V22A/R29H/E35D/D46E/M47I, Q33L/E35D/M43I/L85Q/R94W, H18Y/E35D/V68M/L97Q, Q33L/E35D/M47L/V68M/L85Q/E88D, Q33L/E35D/M43V/M47I/A71G, E35D/M47L/A71G/L97Q, E35D/M47V/A71G/L85M/L97Q, H18Y/Y31H/E35D/M47V/A71G/L85Q, E35D/D46E/M47V/L97Q, E35D/D46V/M47I/A71G/F92V, E35D/M47V/T62A/A71G/V83A/Y87H/L97M, Q33L/E35D/N48K/L85Q/L97Q, E35D/L85Q/K93T/E95V/L97Q, E35D/M47V/N48K/V68M/K89N, Q33L/E35D/M47I/N48D/A71G, Q27H/E35D/M47I/L85Q/D90G, E35D/M47I/L85Q/D90G, E35D/M47I/T62S/L85Q, A26E/E35D/M47L/A71G, E35D/M47I/Y87Q/K89E, V22A/E35D/M47I/Y87N, H18Y/A26E/E35D/M47L/L85Q/D90G, E35D/M47L/A71G/L85Q’ E35D/M47V/A71G/E88D, E35D/A71G, E35D/M47V/A71G, I30V/E35D/M47V/A71G/A91V, V22D/E35D/M47L/L85Q, H18Y/E35D/N48K, E35D/T41S/M47V/A71G/K89N, E35D/M47V/N48T/L85Q, E35D/D46E/M47V/A71D/D90G, E35D/T41S/M43I/A71G/D90G, E35D/T41S/M43I/M47V/A71G, E35D/T41S/M43I/M47L/A71G, H18Y/V22A/E35D/M47V/T62S/A71G, H18Y/A26E/E35D/M47L/V68M/A71G/D90G, E35D/K37E/M47V/N48D/L85Q/D90N, Q27H/E35D/D46V/M47L/A71G, V22L/Q27H/E35D/M47I/A71G, E35D/D46V/M47L/V68M/L85Q/E88D, E35D/T41S/M43V/M47I/L70M/A71G, E35D/D46E/M47V/N63D/L85Q, E35D/D46E/M47V/V68M/D90G/K93E, E35D/M43I/M47V/K89N,

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R29C/E35D/M47L/V68M/A71G/L85M, S21P/E35D/K37E/D46E/M47I/V68M,
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H18Y/A26P/E35D/M47Y/V68I/A71G/D90G, H18Y/A26Q/E35D/M47T/V68M/A71G/D90G,
H18R/A26P/E35D/D46N/M47V/V68M/A71G/D90P, or
H18F/A26D/E35D/D46E/M47T/V68M/A71G/D90G, with reference to the numbering of

positions set forth in SEQ ID NO:2~~Q27H/T41S/A71D, I30T/L70R, T13R/C16R/L70Q/A71D, T57I, M43I/C82R, V22L/M38V/M47T/A71D/L85M, I30V/T57I/L70P/A71D/A91T, V22I/L70M/A71D, N55D/K86M, L72P/T79I, L70P/F92S, T79P, E35D/M47I/L65P/D90N, L25S/E35D/M47I/D90N, S44P/I67T/P74S/E81G/E95D, A71D, T13A/I61N/A71D, E81K, A12V/M47V/L70M, K34E/T41A/L72V, T41S/A71D/V84A, E35D/A71D, E35D/M47I, K36R/G78A, Q33E/T41A, M47V/N48H, M47L/V68A, S44P/A71D, Q27H/M43I/A71D/R73S, E35D/T57I/L70Q/A71D, M47I/E88D, M42I/I61V/A71D, P51A/A71D, H18Y/M47I/T57I/A71G, V20I/M47V/T57I/V84I, V20I/M47V/A71D, A71D/L72V/E95K, E35D/A71D, E35D/I67L/A71D, T13R/M42V/M47I/A71D, E35D, E35D/M47I/L70M, E35D/A71D/L72V, E35D/M43L/L70M, A26P/E35D/M43I/L85Q/E88D, E35D/D46V/L85Q, M47V/I69F/A71D/V83I, H18Y/A26T/E35D/A71D/L85Q, E35D/M47L, E23D/M42V/M43I/I58V/L70R, V68M/L70M/A71D/E95K, N55I/T57I/I69F, E35D/M43I/A71D, T41S/T57I/L70R, V20I/A71D, E23G/A26S/E35D/T62N/A71D/L72V/L85M, V22L/E35D/M43L/A71G/D76H, A26E/E35D/M47L/L85Q, D46E/A71D, Y31H/E35D/T41S/V68L/K93R/R94W, A26E/Q33R/E35D/M47L/L85Q/K86E, A26E/Q33R/E35D/M47L/L85Q, E35D/M47L/L85Q, A26E/Q33L/E35D/M47L/L85Q, A26E/Q33L/E35D/M47L, H18Y/A26E/Q33L/E35D/M47L/L85Q, Q33L/E35D/M47I, H18Y/Q33L/E35D/M47I, Q33L/E35D/D46E/M47I, Q33R/E35D/D46E/M47I, H18Y/E35D/M47L, Q33L/E35D/M47V, Q33L/E35D/M47V/T79A, Q33L/E35D/T41S/M47V, Q33L/E35D/M47I/L85Q, Q33L/E35D/M47I/T62N/L85Q, Q33L/E35D/M47V/L85Q, A26E/E35D/M43T/M47L/L85Q/R94Q, Q33R/E35D/K37E/M47V/L85Q, V22A/E23D/Q33L/E35D/M47V, E24D/Q33L/E35D/M47V/K54R/L85Q, S15P/Q33L/E35D/M47L/L85Q, E7D/E35D/M47I/L97Q, Q33L/E35D/T41S/M43I, E35D/M47I/K54R/L85E, Q33K/E35D/D46V/L85Q, Y31S/E35D/M47L/T79L/E88G, H18L/V22A/E35D/M47L/N48T/L85Q, Q27H/E35D/M47L/L85Q/R94Q/E95K, Q33K/E35D/M47V/K89E/K93R, E35D/M47I/E77A/L85Q/R94W, A26E/E35D/M43I/M47L/L85Q/K86E/R94W, Q27H/Q33L/E35D/M47V/N55D/L85Q/K89N, H18Y/V20A/Q33L/E35D/M47V/Y53F, Q33L/E35D/M47L/A71G/F92S, V22A/R29H/E35D/D46E/M47I, Q33L/E35D/M43I/L85Q/R94W, H18Y/E35D/V68M/L97Q, Q33L/E35D/M47L/V68M/L85Q/E88D, Q33L/E35D/M43V/M47I/A71G,~~

~~E35D/M47L/A71G/L97Q, E35D/M47V/A71G/L85M/L97Q,~~
~~H18Y/Y31H/E35D/M47V/A71G/L85Q, E35D/D46E/M47V/L97Q,~~
~~E35D/D46V/M47I/A71G/F92V, E35D/M47V/T62A/A71G/V83A/Y87H/L97M,~~
~~Q33L/E35D/N48K/L85Q/L97Q, E35D/L85Q/K93T/E95V/L97Q,~~
~~E35D/M47V/N48K/V68M/K89N, Q33L/E35D/M47I/N48D/A71G,~~
~~Q27H/E35D/M47I/L85Q/D90G, E35D/M47I/L85Q/D90G, E35D/M47I/T62S/L85Q,~~
~~A26E/E35D/M47L/A71G, E35D/M47I/Y87Q/K89E, V22A/E35D/M47I/Y87N,~~
~~H18Y/A26E/E35D/M47L/L85Q/D90G, E35D/M47L/A71G/L85Q' E35D/M47V/A71G/E88D,~~
~~E35D/A71G, E35D/M47V/A71G, I30V/E35D/M47V/A71G/A91V, V22D/E35D/M47L/L85Q,~~
~~H18Y/E35D/N48K, E35D/T41S/M47V/A71G/K89N, E35D/M47V/N48T/L85Q,~~
~~E35D/D46E/M47V/A71D/D90G, E35D/T41S/M43I/A71G/D90G,~~
~~E35D/T41S/M43I/M47V/A71G, E35D/T41S/M43I/M47L/A71G,~~
~~H18Y/V22A/E35D/M47V/T62S/A71G, H18Y/A26E/E35D/M47L/V68M/A71G/D90G,~~
~~E35D/K37E/M47V/N48D/L85Q/D90N, Q27H/E35D/D46V/M47L/A71G,~~
~~V22L/Q27H/E35D/M47I/A71G, E35D/D46V/M47L/V68M/L85Q/E88D,~~
~~E35D/T41S/M43V/M47I/L70M/A71G, E35D/D46E/M47V/N63D/L85Q,~~
~~E35D/D46E/M47V/V68M/D90G/K93E, E35D/M43I/M47V/K89N,~~
~~E35D/M47L/A71G/L85M/F92Y, V22D/E35D/M47L/L70M/L97Q, E35D/T41S/M47V/L97Q,~~
~~E35D/Y53H/A71G/D90G/L97R, Q33L/E35D/M43I/Y53F/T62S/L85Q,~~
~~E35D/M38T/D46E/M47V/N48S, Q33R/E35D/M47V/N48K/L85M/F92L,~~
~~E35D/M38T/M43V/M47V/N48R/L85Q, T28Y/Q33H/E35D/D46V/M47I/A71G,~~
~~E35D/N48K/L72V, E35D/T41S/N48T, D46V/M47I/A71G, M47I/A71G,~~
~~E35D/M43I/M47L/L85M, E35D/M43I/D46E/A71G/L85M, H18Y/E35D/M47L/A71G/A91S,~~
~~E35D/M47I/N48K/I61F, E35D/M47V/T62S/L85Q, M43I/M47L/A71G, E35D/M47V,~~
~~E35D/M47L/A71G/L85M, V22A/E35D/M47L/A71G, E35D/M47L/A71G, E35D/D46E/M47I,~~
~~Q27H/E35D/M47I, E35D/D46E/L85M, E35D/D46E/A91G, E35D/D46E, E35D/L97R,~~
~~H18Y/E35D, Q27L/E35D/M47V/I61V/L85M, E35D/M47V/I61V/L85M,~~
~~E35D/M47V/L85M/R94Q, E35D/M47V/N48K/L85M, H18Y/E35D/M47V/N48K,~~
~~A26E/Q27R/E35D/M47L/N48Y/L85Q, E35D/D46E/M47L/V68M/L85Q/F92L,~~
~~E35D/M47I/T62S/L85Q/E88D, E24D/Q27R/E35D/T41S/M47V/L85Q,~~

S15T/H18Y/E35D/M47V/T62A/N64S/A71G/L85Q/D90N,
E35D/M47L/V68M/A71G/L85Q/D90G, H18Y/E35D/M47I/V68M/A71G/R94L,
Q33R/M47V/T62N/A71G, H18Y/V22A/E35D/T41S/M47V/T62N/A71G/A91G,
E24D/E35D/M47L/V68M/E95V/L97Q, E35D/D46E/M47I/T62A/V68M/L85M/Y87C,
E35D/D46E/M47I/V68M/L85M, E35D/D46E/M47L/V68M/A71G/Y87C/K93R,
E35D/D46E/M47L/V68M/T79M/L85M, E35D/D46E/M47L/V68M/T79M/L85M/L97Q,
E35D/D46E/M47V/V68M/L85Q, E35D/M43I/M47L/V68M, E35D/M47I/V68M/Y87N,
E35D/M47L/V68M/E95V/L97Q, E35D/M47L/Y53F/V68M/A71G/K93R/E95V,
E35D/M47V/N48K/V68M/A71G/L85M, E35D/M47V/N48K/V68M/L85M,
E35D/M47V/V68M/L85M, E35D/M47V/V68M/L85M/Y87D,
E35D/T41S/D46E/M47I/V68M/K93R/E95V, H18Y/E35D/D46E/M47I/V68M/R94L,
H18Y/E35D/D46E/M47I/V68M/R94L, H18Y/E35D/M38I/M47L/V68M/L85M,
H18Y/E35D/M47I/V68M/Y87N, H18Y/E35D/M47I/V68M/Y87N,
H18Y/E35D/M47L/V68M/A71G/L85M, H18Y/E35D/M47L/V68M/A71G/L85M,
H18Y/E35D/M47L/V68M/E95V/L97Q, H18Y/E35D/M47L/V68M/E95V/L97Q,
H18Y/E35D/M47L/Y53F/V68M/A71G, H18Y/E35D/M47L/Y53F/V68M/A71G,
H18Y/E35D/M47L/Y53F/V68M/A71G/K93R/E95V,
H18Y/E35D/M47L/Y53F/V68M/A71G/K93R/E95V, H18Y/E35D/M47V/V68M/L85M,
H18Y/E35D/M47V/V68M/L85M, H18Y/E35D/V68M/A71G/R94Q/E95V,
H18Y/E35D/V68M/A71G/R94Q/E95V, H18Y/E35D/V68M/L85M/R94Q,
H18Y/E35D/V68M/L85M/R94Q, H18Y/E35D/V68M/T79M/L85M,
H18Y/V22D/E35D/M47V/N48K/V68M, Q27L/Q33L/E35D/T41S/M47V/N48K/V68M/L85M,
Q33L/E35D/M47V/T62S/V68M/L85M, Q33R/E35D/M38I/M47L/V68M,
R29C/E35D/M47L/V68M/A71G/L85M, S21P/E35D/K37E/D46E/M47I/V68M,
S21P/E35D/K37E/D46E/M47I/V68M/R94L, T13R/E35D/M47L/V68M,
T13R/Q27L/Q33L/E35D/T41S/M47V/N48K/V68M/L85M,
T13R/Q33L/E35D/M47L/V68M/L85M, T13R/Q33L/E35D/M47V/T62S/V68M/L85M,
T13R/Q33R/E35D/M38I/M47L/V68M, T13R/Q33R/E35D/M38I/M47L/V68M/E95V/L97Q,
T13R/Q33R/E35D/M38I/M47L/V68M/L85M,
T13R/Q33R/E35D/M38I/M47L/V68M/L85M/R94Q, T13R/Q33R/E35D/M47L/V68M,

~~T13R/Q33R/E35D/M47L/V68M/L85M, V22D/E24D/E35D/M47L/V68M, V22D/E24D/E35D/M47L/V68M/L85M/D90G, V22D/E24D/E35D/M47V/V68M, H18Y/E35D/M47V/V68M/A71G, H18C/A26P/E35D/M47L/V68M/A71G, H18I/A26P/E35D/M47V/V68M/A71G, H18L/A26N/D46E/V68M/A71G/D90G, H18L/E35D/M47V/V68M/A71G/D90G, H18T/A26N/E35D/M47L/V68M/A71G, H18V/A26K/E35D/M47L/V68M/A71G, H18V/A26N/E35D/M47V/V68M/A71G, H18V/A26P/E35D/M47V/V68L/A71G, H18V/A26P/E35D/M47L/V68M/A71G, H18V/E35D/M47V/V68M/A71G/D90G, H18Y/A26P/E35D/M47I/V68M/A71G, H18Y/A26P/E35D/M47V/V68M/A71G, H18Y/E35D/M47V/V68L/A71G/D90G, H18Y/E35D/M47V/V68M/A71G/D90G, A26P/E35D/M47I/V68M/A71G/D90G, H18V/A26G/E35D/M47V/V68M/A71G/D90G, H18V/A26S/E35D/M47L/V68M/A71G/D90G, H18V/A26R/E35D/M47L/V68M/A71G/D90G, H18V/A26D/E35D/M47V/V68M/A71G/D90G, H18V/A26Q/E35D/M47V/V68L/A71G/D90G, H18A/A26P/E35D/M47L/V68M/A71G/D90G, H18A/A26N/E35D/M47L/V68M/A71G/D90G, H18F/A26P/E35D/M47I/V68M/A71G/D90G, H18F/A26H/E35D/M47L/V68M/A71G/D90G, H18F/A26N/E35D/M47V/V68M/A71G/D90K, H18Y/A26N/E35D/M47F/V68M/A71G/D90G, H18Y/A26P/E35D/M47Y/V68I/A71G/D90G, H18Y/A26Q/E35D/M47T/V68M/A71G/D90G, H18R/A26P/E35D/D46N/M47V/V68M/A71G/D90P, or H18F/A26D/E35D/D46E/M47T/V68M/A71G/D90G, with reference to the numbering of positions set forth in SEQ ID NO: 2~~.

7. The purified CD80-Fc fusion protein of any of claims 1-6, wherein the variant CD80 polypeptide comprises the amino acid modifications E35D/M47I/L70M.

8. The purified CD80-Fc fusion protein of any of claims 1-6, wherein the variant CD80 polypeptide comprises the amino acid modifications E35D/M47V/N48K/V68M/K89N.

9. The purified CD80-Fc fusion protein of any of claims 1-6, wherein the variant CD80 polypeptide comprises the amino acid modifications H18Y/A26E/E35D/M47L/V68M/A71G/D90G.

10. The purified CD80-Fc fusion protein of any of claims 1-6, wherein the variant CD80 polypeptide comprises the amino acid modifications E35D/D46E/M47V/V68M/D90G/K93E.

11. The purified CD80-Fc fusion protein of any of claims 1-6, wherein the variant CD80 polypeptide comprises the amino acid modifications E35D/D46V/M47L/V68M/L85Q/E88D.

12. The purified CD80-Fc fusion protein of any of claims 1-11, wherein the unmodified CD80 comprises (i) a portion of the sequence of amino acids set forth in SEQ ID NO:2 comprising an IgV domain or (ii) a sequence of amino acids that has at least 95% sequence identity to a portion of the sequence of amino acids of SEQ ID NO:2 comprising the IgV domain.

13. The purified CD80-Fc fusion protein of any of claims 1-12, wherein the IgV domain of the unmodified CD80 polypeptide has the sequence of amino acids 35-135, 35-138, 37-138 or 35-141 of SEQ ID NO:1.

14. The purified CD80-Fc fusion protein of any of claims 1-13, wherein the variant CD80 polypeptide comprises up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acid modifications.

15. The purified ~~CD80~~ CD80-Fc fusion protein of any of claims 1-14, comprising: the sequence of amino acids set forth in any of SEQ ID NOS: 93, 94, 102, 103, 111, 120, 121, 124-130, 132, 133, 134, 138, 142, 143, 144, 147, 149, 176, 177, 185, 194, 195, 198-203, 205, 207-208, 212, 216, 218, 221, 223, 2105-2234, 2236-2251, 2253-2270, 2273-2282, 2284-2286, 2288-2290, 2293-2296, 2508, 2509, 2512-2516, 2518-2540, 2543-2601, 2606, 2607, 2608, 2615-2618, 2623, 2624, 2625, 2630, 2631, 2638-2643, 2648, 2649, 2650, 2652-2658, 2660-2664, 2667, 2668, 2669, 2671-2678, 2682, 2683, 2684, 2688-2693, 2698-2702, 2706-2711, 2717-2718, 2719- 2720, 2721, 2723-2727, 2729-2751, 2754-2812, 2817, 2818, 2819, 2826, 2827, 2828,

2829, 2834, 2835, 2836, 2841, 2842, 2849-2854, 2859, 2860, 2861, 2863-2869, 2871-2875, 2878, 2879, 2880, 2882-2889, 2893, 2894, 2895, 2899-2904, 2909-2913, 2917-2922, 2928, 2929, 2961-2963, 2965-2994, 2996-3022, or a sequence of amino acids that exhibits at least 95% sequence identity to any of SEQ ID NOS: 93, 94, 102, 103, 111, 120, 121, 124-130, 132, 133, 134, 138, 142, 143, 144, 147, 149, 176, 177, 185, 194, 195, 198-203, 205, 207-208, 212, 216, 218, 221, 223, 2105-2234, 2236-2251, 2253-2270, 2273-2296, 2508, 2509, 2512-2516, 2518-2540, 2543-2601, 2606, 2607, 2608, 2615-2618, 2623, 2624, 2625, 2630, 2631, 2638-2643, 2648, 2649, 2650, 2652-2658, 2660-2664, 2667, 2668, 2669, 2671-2678, 2682, 2683, 2684, 2688-2693, 2698-2702, 2706-2711, 2717-2718, 2719- 2720, 2721, 2723-2727, 2729-2751, 2754-2812, 2817, 2818, 2819, 2826, 2827, 2828, 2829, 2834, 2835, 2836, 2841, 2842, 2849-2854, 2859, 2860, 2861, 2863-2869, 2871-2875, 2878, 2879, 2880, 2882-2889, 2893, 2894, 2895, 2899-2904, 2909-2913, 2917-2922, 2928, 2929, 2961-2963, 2965-2994, 2996-3022, and that contains the one or more of the amino acid modifications thereof~~the sequence of amino acids set forth in any of SEQ ID NOS:77-149, 151-223, 2105- 2296, 2508-2929, or 2961-3022, or a sequence of amino acids that exhibits at least 95% sequence identity to any of SEQ ID NOS: 77-149, 151-223, 2105- 2296, 2508-2929, or 2961-3022, and that contains the one or more of the amino acid modifications thereof~~.

16. The purified CD80 Fc-fusion protein of any of claims 1-15, comprising the sequence of amino acids set forth in any of SEQ ID NOS:199, 208, 2250, 2276, 2280, or 2284.

17. A purified CD80-Fc fusion protein comprising the formula CD80-linker-Fc, wherein the CD80 is a variant CD80 polypeptide comprising the sequence of amino acids set forth in SEQ ID NO:199.

18. A purified CD80-Fc fusion protein comprising the formula CD80-linker-Fc, wherein the CD80 is a variant CD80 polypeptide comprising the sequence of amino acids set forth in SEQ ID NO:208.

19. A purified CD80-Fc fusion protein comprising the formula CD80-linker-Fc, wherein the CD80 is a variant CD80 polypeptide comprising the sequence of amino acids set forth in SEQ ID NO:2250.

20. A purified CD80-Fc fusion protein comprising the formula CD80-linker-Fc, wherein the CD80 is a variant CD80 polypeptide comprising the sequence of amino acids set forth in SEQ ID NO:2276.

21. A purified CD80-Fc fusion protein comprising the formula CD80-linker-Fc, wherein the CD80 is a variant CD80 polypeptide comprising the sequence of amino acids set forth in SEQ ID NO:2280.

22. A purified CD80-Fc fusion protein comprising the formula CD80-linker-Fc, wherein the CD80 is a variant CD80 polypeptide comprising the sequence of amino acids set forth in SEQ ID NO:2284.

23. The purified CD80-Fc fusion protein of any of claims 1-22, wherein the Fc domain is a variant Fc domain with reduced effector function.

24. The purified CD80-Fc fusion protein of claim 23, wherein the Fc domain is a variant human IgG1 Fc domain with reduced effector function.

25. The purified CD80-Fc fusion protein of any of claims 1-24, wherein the Fc domain comprises one or more amino acid modifications selected from among E233P, L234A, L234V, L235A, L235E, G236del, G237A, S267K, N297G, V302C, and K447del each by EU numbering.

26. The purified CD80-Fc fusion protein of any of claims 1-25, wherein the Fc domain comprises the amino acid substitution N297G by EU numbering.

27. The purified CD80-Fc fusion protein of any of claims 1-25, wherein the Fc domain comprises the amino acid substitutions R292C/N297G/V302C by EU numbering.

28. The purified CD80-Fc fusion protein of any of claims 1-25, wherein the Fc domain comprises the amino acid substitutions L234A/L235E/G237A by EU numbering.

29. The purified CD80-Fc fusion protein of any of claims 1-28, wherein the Fc domain comprises the amino acid substitution C220S by EU numbering and/or wherein the Fc domain comprises K447del by EU numbering.

30. The purified CD80-Fc fusion protein of any one of claims 1-29, wherein the Fc domain comprises the sequence of amino acids set forth in any of SEQ ID NOS:356-358, 376, and 1712-1715 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NOS: 356-358, 376, and 1712-1715 and exhibits reduced effector function.

31. The purified CD80-Fc fusion protein of any one of claims 1-29, wherein the Fc domain comprises the sequence of amino acids set forth in SEQ ID NO:1712 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:1712 and exhibits reduced effector function.

32. The purified CD80-Fc fusion protein of any one of claims 1-29, wherein the Fc domain comprises the sequence of amino acids set forth in SEQ ID NO:1713 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:1713 and exhibits reduced effector function.

33. The purified CD80-Fc fusion protein of any one of claims 1-29, wherein the Fc domain comprises the sequence of amino acids set forth in SEQ ID NO:1714 or a sequence of

amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:1714 and exhibits reduced effector function.

34. The purified CD80-Fc fusion protein of any one of claims 1-29, wherein the Fc domain comprises the sequence of amino acids set forth in SEQ ID NO:1715 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:1715 and exhibits reduced effector function.

35. The purified CD80-Fc fusion protein of any of claims 1-34, wherein the variant CD80 polypeptide is linked to the Fc domain indirectly via a linker.

36. The purified CD80-Fc fusion protein of any one of claims 17-22 and 35, wherein the linker has 1-20 amino acid residues.

37. The purified CD80-Fc fusion protein of claim 35 or claim 36, wherein the linker is GGGGS (SEQ ID NO:1717).

38. The purified CD80-Fc fusion protein of any one of claims 35-37, wherein the linker is GSGGGGS (SEQ ID NO:1716).

39. A purified immunomodulatory protein that is a dimer comprising two CD80-Fc fusion proteins of any of claims 1-38.

40. The purified immunomodulatory protein of claim 39, wherein the dimer is a homodimer.

41. A method of producing the purified CD80-Fc fusion protein of any of claims 1-38 or the purified immunomodulatory protein of claim 39 or claim 40, comprising introducing a

nucleic acid molecule encoding a CD80-Fc fusion protein or a vector comprising the nucleic acid into a host cell under conditions to express the protein in the cell, and further comprising isolating or purifying the CD80-Fc fusion protein from the cell.

42. A pharmaceutical composition, comprising the CD80-Fc fusion protein of any of claims 1-38 or the immunomodulatory protein of claim 39 or claim 40, and a pharmaceutically acceptable excipient.

~~43. Use of the pharmaceutical composition of claim 42 in the manufacture of a medicament for modulating an immune response in a subject, optionally wherein the immune response is increased.~~

434. Use of the pharmaceutical composition of claim 42 in the manufacture of a medicament for treating a tumor or cancer in a subject in need thereof.