

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 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 exhibits increased binding affinity to the ectodomain of human PD-L1 compared to the binding affinity of the unmodified 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.

[0005d] 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:2250.

[0005e] 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:2276.

[0005f] 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:2280.

[0005g] 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:2284.

[0006] In some examples, the variant CD80 polypeptide contains one or more amino acid substitutions, at one or more positions, in an unmodified CD80 or specific binding fragment thereof, selected from among E7D, E23D, E23G, A26E, A26P, A26S, A26T, I30F, I30T, I30V, K34E, E35D, E35G, D46E, D46V, P51A, N55D, N55I, T57A, T57I, I58V, L65P, A71D, A71G, R73H, R73S, G78A, T79A, T79I, T79L, T79M, T79P, C82R, V84A, and V84I, where the position(s) of the amino acid modification(s) correspond(s) to the numbering of positions of CD80 set forth in SEQ ID NO: 2.

[0007] In some examples, the provided variant CD80 polypeptide contains one or more further modifications at one or more positions corresponding to position(s) 7, 12, 13, 15, 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, 74, 76, 77, 78, 79, 81, 82, 83, 84, 85, 86, 87, 88, 90, 91, 92, 93, 94, 95, and/or 97 with reference to numbering of SEQ ID NO: 2. In some examples, the further modifications include one or more amino acid substitutions in the CD80 or specific binding fragment thereof, selected from among E7D, T13A, T13R, S15P, S15T, C16R, V20A, V20I, V22D, V22I, V22L, E23D, E23G, E24D, L25S, A26E, A26P, A26S, A26T, Q27H, Q27L, I30F, I30T, I30V, Y31S, Q33E, Q33K, Q33L, Q33R, K34E, E35D, E35G, K36R, T41S, M42I, M42V, M43L, M43T, D46E, D46V, M47I, M47L, M47V, N48H, N48D, N48H, N48K, N48R, N48S, N48T, N48Y, P51A, Y53F, K54E, K54N, K54R, N55D, N55I, T57A, T57I, I58V, I61F,

I61V, T62A, T62N, L65P, I67L, I67V, V68E, V68L, I69F, L70M, L70P, L70Q, 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, E88V, F92S, F92V, R94Q, R94W, E95D, E95V, L97M, and L97Q where the position(s) of the amino acid substitution(s) correspond(s) to the numbering of positions of CD80 set forth in SEQ ID NO: 2.

[0008] In some examples, the one or more amino acid substitution is selected from among: I30F/L70P, 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/L70P/E77G, T57A/I69T, N55D/K86M, L72P/T79I, T79P, E35D/M47I/L65P/D90N, L25S/E35D/M47I/D90N, A71D, T13A/I61N/A71D, K34E/T41A/L72V, T41S/A71D/V84A, E35D/A71D, E35D/M47I, K36R/G78A, S44P/A71D, Q27H/M43I/A71D/R73S, Q33R/K54N/T57I/I67V/A71D, E35D/T57I/L70Q/A71D, M42I/I61V/A71D, P51A/A71D, H18Y/M47I/T57I/A71G, V20I/M47V/T57I/V84I, V20I/M47V/A71D, A71D/L72V/E95K, V22L/E35G/A71D/L72P, E35D/A71D, E35D/I67L/A71D, Q27H/E35G/A71D/L72P/T79I, T13R/M42V/M47I/A71D, E35D, E35D/M47I/L70M, E35D/A71D/L72V, E35D/M43L/L70M, A26P/E35D/M43I/L85Q/E88D, E35D/D46V/L85Q, Q27L/E35D/M47I/T57I/L70Q/E88D, M47V/I69F/A71D/V83I, E35D/T57A/A71D/L85Q, 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, H18Y/A71D/L72P/E88V, V20I/A71D, E23G/A26S/E35D/T62N/A71D/L72V/L85M, A12T/E24D/E35D/D46V/I61V/L72P/E95V, V22L/E35D/M43L/A71G/D76H, E35G/K54E/A71D/L72P, L70Q/A71D, 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/M47V/N48K/V68M/L85Q, E35D/M47V/N48K/V68M/K89N, E35D/M47V/N48K/L85M, E35D/M47V/N48K/K89N, E35D/M47V/I61V/L85M, E35D/M47V/T62S/L85Q, E35D/M47V/V68M, E35D/M47V/V68M/L85M, E35D/M47V/V68M/L85M/Y87D, E35D/M47V/V68M/L85Q/K89N, E35D/M47V/V68M/K89N, E35D/M47V/L85M/R94Q, E35D/M47V/K89N, E35D/N48K, E35D/N48K/V68M, E35D/N48K/V68M/K89N, E35D/N48K/L72V, E35D/N48K/K89N, E35D/V68M, E35D/V68M/A71G/D90G, E35D/V68M/L85Q, E35D/V68M/K89N, E35D/L85Q, E35D/K89N, E35D/L97R, M43I/M47L/A71G, D46V, D46V/M47I/A71G, D46V/M47L, D46V/M47L/V68M, D46V/M47L/V68M/L85Q, D46V/M47L/L85Q, D46V/V68M, D46V/V68M/L85Q, D46V/L85Q, M47I/A71G, M47L, M47L/V68M, M47L/V68M/A71G/D90G, M47L/V68M/L85Q, M47L/L85Q, M47V, M47V/N48K, M47V/N48K/V68M, M47V/N48K/V68M/K89N, M47V/N48K/K89N, M47V/V68M, M47V/V68M/K89N, M47V/K89N, N48K, N48K/V68M, N48K/V68M/K89N, N48K/K89N, V68M, V68M/L85Q, V68M/K89N, L85Q, K89N, and delE10-A98, where the position(s) of the amino acid substitution(s) correspond(s) to the positions of CD80 set forth in SEQ ID NO: 2.

[0009] 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 substitutions in an unmodified CD80 or specific binding fragment thereof, selected from among E7D, T13A, T13R, S15P, S15T, C16R, V20A, V20I, V22D, V22I, V22L, E23D, E23G, E24D, L25S, A26E, A26P, A26S, A26T, Q27H, Q27L, T28Y, I30F, I30T, I30V, Y31C, Y31S, Q33E, Q33K, Q33L, Q33R, K34E, E35D, E35G, K36R, T41S, M42I, M42V, M43L, M43T, D46E, D46V, M47I, M47L, M47V, N48D, N48H, N48K, N48R, N48S, N48T, N48Y, P51A, Y53F, Y53H, K54E, K54N, K54R, N55D, N55I, T57A, T57I, I58V, I61V, T62A, T62N, N63D, L65P, I67L, I67V, V68E, V68L, I69F, L70M, L70P, L70Q, 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, F92S, F92V, R94Q, R94W, E95D, E95V, L97M, and L97Q, where the position(s) of the amino acid substitution(s) correspond(s) to the numbering of positions of CD80 set forth in SEQ ID NO: 2.

[0010] In some examples, the provided variant CD80 polypeptide contains one or more further modifications at one or more positions corresponding to position(s) 7, 12, 13, 15, 16, 18, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 41, 42, 43, 44, 46, 47, 48, 51, 53,

54, 55, 57, 58, 61, 62, 63, 65, 67, 68, 69, 70, 71, 72, 73, 74, 76, 77, 78, 79, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, and/or 97 with reference to numbering of SEQ ID NO: 2. In some examples, the further modifications include one or more amino acid substitutions in the CD80 or specific binding fragment thereof, selected from among E7D, A12T, A12V, T13A, T13R, S15P, S15T, C16R, H18L, H18Y, V20A, V20I, V22A, V22D, V22I, V22L, E23D, E23G, E24D, L25S, A26E, A26P, A26S, A26T, Q27H, Q27L, T28Y, R29H, I30F, I30T, I30V, Y31C, Y31H, Y31S, Q33E, Q33H, Q33K, Q33L, Q33R, K34E, E35D, E35G, K36R, K37E, M38T, M38V, T41A, T41S, M42I, M42V, M43I, M43L, M43T, M43V, S44P, D46E, D46V, M47I, M47L, M47T, M47V, N48D, N48H, N48K, N48R, N48S, N48T, N48Y, P51A, K54E, Y53F, Y53H, K54R, N55D, N55I, T57A, T57I, I58V, I61F, I61N, I61V, T62A, T62N, T62S, N63D, L65P, I67L, I67T, V68A, V68L, V68M, I69F, I69T, L70M, L70P, L70Q, L70R, A71D, A71G, L72P, L72V, R73S, P74S, D76H, E77GE77A, G78A, T79A, T79I, T79L, T79M, T79P, E81G, E81K, C82R, V83A, V83I, V84A, V84I, L85E, L85M, L85Q, K86E, K86M, Y87C, Y87D, Y87H, Y87N, Y87Q, E88D, E88V, E88G, K89E, K89N, D90G, D90N, A91S, A91T, A91V, F92L, F92S, F92V, F92Y, K93E, K93R, K93T, R94Q, R94W, E95D, E95K, E95V, L97M, L97Q, E95V, and L97R where the position(s) of the amino acid substitution(s) correspond(s) to the numbering of positions of CD80 set forth in SEQ ID NO: 2.

[0011] In some examples, the one or more amino acid substitution is selected from among: I30F/L70P, 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/L70P/E77G, T57A/I69T, N55D/K86M, L72P/T79I, L70P/F92S, T79P, E35D/M47I/L65P/D90N, L25S/E35D/M47I/D90N, A71D, E81K/A91S, 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, V22L/E35G/A71D/L72P, E35D/A71D, E35D/I67L/A71D, Q27H/E35G/A71D/L72P/T79I, T13R/M42V/M47I/A71D, E35D, E35D/M47I/L70M, E35D/A71D/L72V, E35D/M43L/L70M, A26P/E35D/M43I/L85Q/E88D, E35D/D46V/L85Q, Q27L/E35D/M47I/T57I/L70Q/E88D, M47V/I69F/A71D/V83I, E35D/T57A/A71D/L85Q, 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, H18Y/A71D/L72P/E88V, V20I/A71D,

A26E/E35D/M47L/V68M/A71G/D90G, H18Y/E35D/M47L/V68M/A71G/D90G, H18Y/A26E/M47L/V68M/A71G/D90G, H18Y/A26E/E35D/V68M/A71G/D90G, H18Y/A26E/E35D/M47L/A71G/D90G, H18Y/A26E/E35D/M47L/V68M/D90G, H18Y/A26E/E35D/M47L/V68M/A71G, E35D/M47L/V68M/A71G/D90G, H18Y/M47L/V68M/A71G/D90G, H18Y/A26E/V68M/A71G/D90G, H18Y/A26E/E35D/A71G/D90G, H18Y/A26E/E35D/M47L/D90G, H18Y/A26E/E35D/M47L/V68M, A26E/M47L/V68M/A71G/D90G, A26E/E35D/V68M/A71G/D90G, A26E/E35D/M47L/A71G/D90G, A26E/E35D/M47L/V68M/D90G, A26E/E35D/M47L/V68M/A71G, H18Y/E35D/V68M/A71G/D90G, H18Y/E35D/M47L/A71G/D90G, H18Y/E35D/M47L/V68M/D90G, H18Y/E35D/M47L/V68M/A71G, H18Y/A26E/M47L/A71G/D90G, H18Y/A26E/M47L/V68M/D90G, H18Y/A26E/M47L/V68M/A71G, H18Y/A26E/E35D/V68M/D90G, H18Y/A26E/E35D/V68M/A71G, H18Y/A26E/E35D/M47L/A71G, M47L/V68M/A71G/D90G, H18Y/V68M/A71G/D90G, H18Y/A26E/A71G/D90G, H18Y/A26E/E35D/D90G, H18Y/A26E/E35D/M47L, E35D/V68M/A71G/D90G, E35D/M47L/A71G/D90G, E35D/M47L/V68M/D90G, E35D/M47L/V68M/A71G, A26E/V68M/A71G/D90G, A26E/M47L/A71G/D90G, A26E/M47L/V68M/D90G, A26E/M47L/V68M/A71G, A26E/E35D/A71G/D90G, A26E/E35D/V68M/D90G, A26E/E35D/V68M/A71G, A26E/E35D/M47L/D90G, A26E/E35D/M47L/V68M, H18Y/M47L/A71G/D90G, H18Y/M47L/V68M/D90G, H18Y/M47L/V68M/A71G, H18Y/E35D/A71G/D90G, H18Y/E35D/V68M/D90G, H18Y/E35D/V68M/A71G, H18Y/E35D/M47L/D90G, H18Y/E35D/M47L/A71G, H18Y/E35D/M47L/V68M, H18Y/A26E/V68M/D90G, H18Y/A26E/V68M/A71G, H18Y/A26E/M47L/D90G, H18Y/A26E/M47L/A71G, H18Y/A26E/M47L/V68M, H18Y/A26E/E35D/A71G and H18Y/A26E/E35D/V68M, where the position(s) of the amino acid substitution(s) correspond(s) to the positions of CD80 set forth in SEQ ID NO: 2.

[0012] In some examples, the one or more amino acid substitutions is selected from among V20I, V22I, V22L, A26E, Q27H, Q33L, Q33R, E35D, E35G, T41S, M43L, D46E, D46V, M47I, M47L, M47V, N55D, T57I, I61V, L70M, A71D, A71G, L72V, and L85M, L85Q, R94W, and L97Q, where the position(s) of the amino acid substitution(s) correspond(s) to the positions of CD80 set forth in SEQ ID NO: 2. In some examples, the one or more amino acid

substitutions is selected from among V20I, V22L, A26E, Q27H, Q33L, Q33R, E35D, E35G, M47I, D46E, D46V, M47L, M47V, T57I, L70M, A71D, A71G, L72V, and L85M, L85Q, L97Q, or the one or more amino acid modification is selected from among A26E, Q33L, E35D, M47I, M47L, M47V, T57I, L70M, A71D, A71G, and L85Q; or the one or more amino acid modification is selected from among A26E, E35D, D46V, M47L, M47V, L70M, A71G, and L85Q; or the one or more amino acid modification(s) comprises A26E; or the one or more amino acid modification(s) comprises E35D; or the one or more amino acid modification(s) comprises D46V; or the one or more amino acid modification(s) comprise M47L; or the one or more amino acid modification(s) comprise M47V; or the one or more amino acid modification(s) comprise A71G, where the position(s) of the amino acid substitution(s) correspond(s) to the numbering of positions of CD80 set forth in SEQ ID NO: 2. In some examples, the one or more amino acid substitutions is selected from among A26E, Q33L, E35D, M47I, M47L, M47V, T57I, L70M, A71D, A71G, and L85Q, where the position(s) of the amino acid substitution(s) correspond(s) to the positions of CD80 set forth in SEQ ID NO: 2.

[0013] 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 comprises amino acid modifications in an unmodified CD80 or specific binding fragment thereof, wherein the amino acid modifications comprise E35D/D46E, E35D/D46V, E35D/M47I, E35D/M47L, E35D/M47V, E35D/V68M, E35D/A71G or E35D/D90G; D46E/M47I, D46E/M47L, D46E/M47V, D46E/V68M, D46E/A71G or D46E/D90G; M47I/V68M, M47I/A71G, M47I/D90G; M47L/V68M, M47L/A71G, M47L/D90G; M47V/V68M, M47V/A71G, M47V/D90G; V68M/A71G or V68M/D90G; A71G/D90G; or E35D/M47I/V68M, E35D/M47L/V68M, E35D/M47V/V68M, wherein the position(s) of the amino acid modification(s) correspond(s) to the numbering of positions of CD80 set forth in SEQ ID NO: 2

[0014] 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 the amino acid substitutions, the one or more amino acid substitutions containing at least the amino acid substitution L70P but not containing the amino acid substitutions V68M, L72P and/or K86E, where the position(s) of the amino acid substitution(s) correspond(s) to the positions of CD80 set forth in SEQ ID NO: 2. In some examples, the one or more amino acid substitution is

selected from among: L70P, I30F/L70P, I30V/T57I/L70P/A71D/A91T, N55D/L70P/E77G, and L70P/F92S.

[0015] In some examples, the unmodified CD80 is a mammalian CD80. In some examples, the CD80 is a human CD80.

[0016] In some examples, the variant CD80 polypeptide contains: the IgV domain or a specific binding fragment thereof; and the IgC domain or a specific binding fragment thereof.

[0017] In some examples, the unmodified CD80 contains (i) the sequence of amino acids set forth in SEQ ID NO:2, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:2; or (iii) is a portion thereof containing an IgV domain or IgC domain or specific binding fragments thereof. In some examples, the specific binding fragment of the IgV domain or the IgC domain has a length of at least 50, 60, 70, 80, 90, 100, 110 or more amino acids; the specific binding fragment of the IgV domain contains a length that is at least 80% of the length of the IgV domain set forth as amino acids 35-135, 35-138, 37-138 or 35-141 of SEQ ID NO:1; or the specific binding fragment of the IgC domain contains a length that is at least 80% of the length of the IgC domain set forth as amino acids 145-230, 154-232, or 142-232 of SEQ ID NO:1.

[0018] In some examples, the variant CD80 polypeptide 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, optionally amino acid substitutions, insertions and/or deletions. In some examples, the variant CD80 polypeptide contains 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: 2, or a specific binding fragment thereof.

[0019] In some examples, the variant CD80 polypeptide exhibits altered binding to the ectodomain of CTLA-4, PD-L1 and/or CD28 compared to the binding of the unmodified CD80 for the ectodomain of CTLA-4, PD-L1 and/or CD28. In some examples, the altered binding is altered binding affinity and/or altered binding selectivity.

[0020] In some examples, the variant CD80 polypeptide contains the IgV domain or a specific fragment thereof and the IgC domain or a specific fragment thereof. In some examples, the variant CD80 polypeptide contains or consists of the sequence of amino acids set forth in any of SEQ ID NOS: 3-75, 2009-2104, 2297-2507, and 2930-2960 or a specific binding fragment thereof, or a sequence of amino acids that exhibits at least 95% sequence

identity to any of SEQ ID NOS: 3-75, 2009-2104, 2297-2507, and 2930-2960, or a specific binding fragment thereof, that contains the one or more of the amino acid substitutions.

[0021] In some examples, the variant CD80 polypeptide contains the IgV domain or a specific binding fragment thereof. In some examples, the IgV domain or specific binding fragment thereof is the only CD80 portion of the variant CD80 polypeptide.

[0022] In some examples, the variant CD80 polypeptide contains the sequence of amino acids set forth in any of SEQ ID NOS: 77-149, 151-223, 2105-2296, 2508-2929, and 2961-3022, or a specific binding fragment thereof, 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, and 2961-3022, or a specific binding fragment thereof, that contains the one or more of the amino acid substitutions.

[0023] In some examples, the IgC domain or specific binding fragment thereof is the only CD80 portion of the variant CD80 polypeptide.

[0024] In some examples, the variant CD80 exhibits altered binding affinity and/or altered binding selectivity to the ectodomain of CTLA4, PD-L1, or CD28, compared to the binding specificity of the unmodified CD80 for the ectodomain of CTLA4, PD-L1, or CD28. In some examples, the CTLA-4 is a human CTLA-4. In some examples, the CD28 is a human CD28. In some examples, the PD-L1 is a human PD-L1.

[0025] In some examples, the variant CD80 exhibits increased binding affinity to the ectodomain of CTLA4 compared to the binding affinity of the unmodified CD80 for the ectodomain of CTLA4. In some examples, the increased affinity to the ectodomain of CTLA-4 is 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 compared to binding affinity of the unmodified CD80 for the ectodomain of CTLA-4.

[0026] In some examples, the variant CD80 polypeptide contains one or more amino acid modifications in an unmodified CD80 or specific binding fragment thereof, corresponding to position(s) 7, 23, 26, 30, 35, 46, 57, 58, 71, 73, 79, and/or 84, with reference to numbering of SEQ ID NO: 2. In some examples, the variant CD80 polypeptide contains one or more amino acid modifications in an unmodified CD80 or specific binding fragment thereof, selected from among E7D, T13A, T13R, S15T, C16R, V20I, V22D, V22L, E23D, E23G, E24D, A26E, A26P, A26S, A26T, Q27H, Q27L, I30V, Q33L, Q33R, E35D, E35G, T41S, M42V, M43L, M43T, D46E, D46V, M47I, M47L, M47V, N48D, N48H, N48K, N48R, N48S, N48T, N48Y, Y53F,

K54E, K54R, T57A, T57I, I58V, I61F, I61V, T62A, T62N, I67L, V68E, I69F, L70M, A71D, A71G, L72V, R73H, P74S, T79I, T79M, E81G, E81K, V84I, L85M, L85Q, Y87C, Y87D, E88V, F92V, R94Q, R94W, E95D, E95V, and L97Q, wherein the position(s) of the amino acid substitution(s) correspond(s) to the positions of CD80 set forth in SEQ ID NO: 2.

[0027] In any of the provided examples, the CD80 polypeptide can exhibit increased binding affinity to the ectodomain of CD28 compared to the binding affinity of the unmodified CD80 for the ectodomain of CD28. In some of such examples, the increased affinity to the ectodomain of CD28 is 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, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, or 200-fold, compared to binding affinity of the unmodified CD80 for the ectodomain of CD28. In some of such examples, the CD80 polypeptide contains one or more amino acid modifications in an unmodified CD80 or specific binding fragment thereof, corresponding to position(s) 23, 26, 35, 46, 55, 57, 58, 71, 79, and/or 84, with reference to numbering of SEQ ID NO: 2. In some of such examples, the CD80 polypeptide contains one or more amino acid modifications in an unmodified CD80 or specific binding fragment thereof, selected from among T13R, S15T, V20I, V22D, V22L, E23D, E23G, E24D, A26E, A26P, A26S, A26T, Q27H, Q27L, Q33R, E35D, E35G, T41S, M42V, M43L, D46E, D46V, M47I, M47L, M47V, N48K, N48Y, Y53F, K54E, N55I, T57A, T57I, I58V, I61F, I61V, T62A, T62N, I67L, V68E, V68L, I69F, L70M, A71D, A71G, L72V, T79I, T79M, V84I, L85M, L85Q, Y87C, Y87D, E88V, R94Q, R94W, E95V, and L97Q, wherein the position(s) of the amino acid substitution(s) correspond(s) to the positions of CD80 set forth in SEQ ID NO: 2.

[0028] In some examples, the variant CD80 polypeptide exhibits increased binding affinity to the ectodomain of PD-L1 compared to the binding affinity of the unmodified CD80 for the ectodomain of PD-L1. In some of such examples, the increased affinity to the ectodomain of PD-L1 is 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, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, 200-fold, 250-fold, 300-fold, 350-fold, 400-fold, or 450-fold compared to binding affinity of the unmodified CD80 for the ectodomain of PD-L1. In some of such examples, the CD80 polypeptide contains one or more amino acid modifications 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 CD80 polypeptide contains one or more amino acid modifications in an

unmodified CD80 or specific binding fragment thereof, selected from among E7D, T13A, T13R, S15T, C16R, V20A, V20I, V22D, V22I, V22L, E23D, E23G, E24D, L25S, A26E, A26P, A26S, A26T, Q27H, Q27L, I30T, I30V, Q33E, Q33K, Q33L, Q33R, K34E, E35D, K36R, T41S, M42I, M42V, M43L, M43T, D46E, D46V, M47I, M47L, M47V, N48D, N48H, N48K, N48R, N48S, N48T, N48Y, P51A, Y53F, K54R, N55D, N55I, T57I, I58V, I61F, I61V, T62A, T62N, L65P, I67L, V68L, I69F, L70M, A71D, A71G, L72V, R73S, P74S, D76H, G78A, T79A, T79I, T79L, T79M, T79P, E81G, E81K, C82R, V84A, V84I, L85E, L85M, L85Q, K86M, Y87C, Y87D, F92S, F92V, R94Q, R94W, E95D, E95V, L97M, and L97Q, wherein the position(s) of the amino acid substitution(s) correspond(s) to the positions of CD80 set forth in SEQ ID NO: 2.

[0029] In some examples, the variant CD80 exhibits decreased binding affinity to the ectodomain of CD28 compared to the binding affinity of the unmodified CD80 for the ectodomain of CD28. In some examples, the decreased affinity to the ectodomain of CD28 is 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 compared to binding affinity of the unmodified CD80 for the ectodomain of CD28.

[0030] In some examples, the variant CD80 polypeptide specifically binds to the ectodomain of CTLA-4 with increased selectivity compared to the unmodified CD80 for the ectodomain of CTLA-4. In some of such examples, the increased selectivity includes a greater ratio of binding of the variant polypeptide for CTLA-4 versus CD28 compared to the ratio of binding of the unmodified CD80 polypeptide for CTLA-4 versus CD28. In some of such examples, the ratio of binding CTLA-4 versus CD28 is greater by at least or at least about 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold or more.

[0031] In any of the provided examples, the variant CD80 polypeptide specifically binds to the ectodomain of PD-L1 with increased selectivity compared to the unmodified CD80 of the ectodomain of PD-L1. In some of such examples, the increased selectivity includes a greater ratio of binding of the variant polypeptide for PD-L1 versus CD28 compared to the ratio of binding of the unmodified CD80 polypeptide for PD-L1 versus CD28. In some of such examples, the ratio is greater by at least or at least about 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold or more.

[0032] In some examples, the variant CD80 polypeptide is a soluble protein. In some examples, the variant CD80 polypeptide is linked to a multimerization domain. In some

examples, the variant CD80 polypeptide is a multimeric polypeptide, optionally a dimeric polypeptide, containing a first variant CD80 polypeptide linked to a multimerization domain and a second variant CD80 polypeptide linked to a multimerization domain. In some examples, the first variant CD80 polypeptide and the second variant CD80 polypeptide are the same. In some examples, the first variant CD80 polypeptide and the second variant CD80 polypeptide are different.

[0033] In some examples, the multimerization domain is an Fc domain or a variant thereof with reduced effector function. In some examples, the variant CD80 polypeptide is linked to a moiety that increases biological half-life of the polypeptide. In some examples, the variant CD80 polypeptide is linked to an Fc domain or a variant thereof with reduced effector function.

[0034] In some examples, the Fc domain is mammalian, optionally human; or the variant Fc domain contains one or more amino acid modifications compared to an unmodified Fc domain that is mammalian, optionally human. In some examples, the Fc domain or variant thereof contains the sequence of amino acids set forth in SEQ ID NO:277, SEQ ID NO:359, or SEQ ID NO: 1712, or a sequence of amino acids that exhibits at least 85% sequence identity to SEQ ID NO:277, SEQ ID NO:359, or SEQ ID NO: 1712. In some examples, the Fc domain contains 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. In some examples, wherein the Fc region is not a human IgG1 Fc containing the mutations R292C, N297G and V302C (corresponding to R77C, N82G and V87C with reference to wild-type human IgG1 Fc set forth in SEQ ID NO: 277). In some examples, the Fc is not the Fc set forth in SEQ ID NO:356. In some examples, the Fc domain contains the amino acid modification C220S by EU numbering. In some examples, the Fc domain contains the sequence of amino acids set forth in any of SEQ ID NOS: 356-358, 376, and 1713-1715 or a sequence of amino acids that exhibits at least 85% sequence identity to any of SEQ ID NOS: 356-358, 376, and 1713-1715 and exhibits reduced effector function. In some examples, the variant CD80 polypeptide is linked to the multimerization domain or Fc indirectly via a linker, optionally a GSG₄S linker (SEQ ID NO: 1716). In some examples, the linker does not consist of three alanines (AAA).

[0035] In some examples, provided herein is an immunomodulatory protein, containing the any of the variant CD80 polypeptides provided herein and a half-life extending moiety. In

some examples, the half-life extending moiety contains 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. In some examples, the half-life extending moiety is or contains Pro/Ala/Ser (PAS) and the variant CD80 polypeptide is PASylated. In some examples, the half-life extending moiety is or contains a multimerization domain. In some examples, the multimerization domain is selected from an Fc region of an immunoglobulin, a leucine zipper, an isoleucine zipper or a zinc finger.

[0036] In some examples, the immunomodulatory protein is a multimer containing a first variant CD80 polypeptide linked to a first multimerization domain and a second variant CD80 polypeptide linked to a second multimerization domain, wherein the first and second multimerization domains interact to form a multimer containing the first and second variant CD80 polypeptide. In some examples, the multimer is a dimer. In some examples, the first variant CD80 polypeptide and the second variant CD80 polypeptide are the same. In some examples, the dimer is a homodimer. In some examples, the dimer is a heterodimer.

[0036a] In one aspect, the present disclosure refers to a purified immunomodulatory protein that is a dimer comprising two of any of the CD80-Fc fusion proteins disclosed herein.

[0037] In some examples, the multimerization domain is or contains an Fc region of an immunoglobulin. In some examples, the Fc region is of an immunoglobulin G1 (IgG1) or an immunoglobulin G2 (IgG2) protein. In some examples, the immunoglobulin protein is human and/or the Fc region is human. In some examples, the Fc region contains the sequence of amino acids set forth in SEQ ID NO: 278 or a variant thereof that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:278. In some examples, the Fc region contains the sequence of amino acids set forth in SEQ ID NO: 277 or a variant thereof that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:277.

[0038] In some examples, the Fc region exhibits one or more effector functions. In some examples, the immunomodulatory protein exhibits Fc-dependent CD28 costimulation, optionally in a T cell stimulation assay in the presence of antigen presenting cells, optionally wherein the T cells comprise Jurkat cells expressing an IL-2 reporter. In some examples, the Fc region exhibits one or more reduced effector function compared to a wildtype Fc region, optionally wherein the wildtype Fc region is a human Fc of human IgG1. In some examples, the one or more effector function is selected from among antibody dependent cellular

cytotoxicity (ADCC), complement dependent cytotoxicity, programmed cell death and cellular phagocytosis.

[0039] In some examples, the Fc region is a variant Fc region containing one or more amino acid substitutions compared to the wildtype Fc region. In some of such examples, the one or more amino acid substitutions of the variant Fc region are selected from Fc N297G, R292C/N297G/V302C, E233P/L234V/L235A/G236del/S267K or L234A/L235E/G237A, wherein the residue is numbered according to the EU index of Kabat. In some examples, the variant Fc region further contains the amino acid substitution C220S, wherein the residues are numbered according to the EU index of Kabat. In some examples, the Fc region contains the sequence of amino acid sequence set forth in any of SEQ ID NOS: 356-358 or a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 356-358 and contains the amino acid substitutions. In some examples, the Fc region contains K447del, wherein the residue is numbered according to the EU index of Kabat. In some examples, the Fc region contains the sequence of amino acid sequence set forth in any of SEQ ID NOS: 1713-1715 or a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 1713-1715 and contains the amino acid substitutions.

[0040] In some examples, the immunomodulatory protein contains any of the variant CD80 polypeptides provided herein that exhibits increased affinity for PD-L1.

[0041] In some examples, the immunomodulatory protein exhibits PD-L1-dependent CD28 costimulation, optionally in a T cell stimulation assay in the presence of antigen presenting cells expressing PD-L1, optionally wherein the T cells comprise Jurkat cells expressing an IL-2 reporter or primary human T cells producing inflammatory cytokines such as IL-2.

[0042] In some examples of the immunomodulatory protein, the variant CD80 polypeptide is linked, directly or indirectly via a linker, to the multimerization domain. In some of such examples, the linker contains 1 to 10 amino acids. In some examples, the linker is selected from AAA, G4S (SEQ ID NO:1717) or (G4S)₂ (SEQ ID NO:330).

[0043] In some examples, the variant CD80 polypeptide is a transmembrane immunomodulatory protein further containing a transmembrane domain linked to the extracellular domain (ECD) or specific binding fragment thereof of the variant CD80 polypeptide. In some examples, the transmembrane domain contains the sequence of amino

acids set forth as residues 243-263 of SEQ ID NO:1 or a functional variant thereof that exhibits at least 85% sequence identity to residues 243-263 of SEQ ID NO:1. In some examples, the variant CD80 polypeptide further contains a cytoplasmic signaling domain linked to the transmembrane domain. In some examples, the cytoplasmic signaling domain contains the sequence of amino acids set forth as residues 264-288 of SEQ ID NO:1 or a functional variant thereof that exhibits at least 85% sequence identity to residues 254-288 of SEQ ID NO:1.

[0044] In some of any of the provided examples, the variant CD80 polypeptide modulates a response of an immune cell, such as a T cell. In some examples, the response, e.g., T cell response, is increased or is decreased. In some examples, the variant CD80 increases IFN-gamma (interferon-gamma) expression relative to the unmodified CD80 in an *in vitro* primary T-cell assay. In some examples, the variant CD80 decreases IFN-gamma (interferon-gamma) expression relative to the unmodified CD80 in an *in vitro* primary T-cell assay. In some examples of any one of the variant CD80 polypeptides described herein, the variant CD80 polypeptide increases T cell signaling relative to the unmodified CD80, such as determined using a reporter assay involving a T cell (e.g., Jurkat) engineered with a reporter (e.g., luciferase) operably connected to an IL-2 promoter. In some examples of any one of the variant CD80 polypeptides described herein, the variant CD80 polypeptide decreases T cell signaling relative to the unmodified CD80, such as determined using a reporter assay involving a T cell (e.g., Jurkat) engineered with a reporter (e.g., luciferase) operably connected to an IL-2 promoter. In some of any such examples, the variant CD80 polypeptide is provided in any of a variety of formats, such as soluble or immobilized (e.g., plate-bound).

[0045] In some examples, the variant CD80 polypeptide is deglycosylated.

[0046] In some examples, provided herein is an immunomodulatory protein, containing any of the provided variant CD80 polypeptide linked to a second polypeptide containing an immunoglobulin superfamily (IgSF) domain. In some examples, the IgSF domain is affinity modified and exhibits altered binding to one or more of its cognate binding partner(s) compared to the unmodified or wild-type IgSF domain. In some examples, the IgSF domain exhibits increased binding to one or more of its cognate binding partner(s) compared to the unmodified or wild-type IgSF domain.

[0047] In some examples, the variant CD80 is a first CD80 variant polypeptide and the IgSF domain of the second polypeptide is an IgSF domain from a second variant CD80 polypeptide that is any of the variant CD80 polypeptides provided herein, wherein the first and

second CD80 variant are the same or different. In some examples, the variant CD80 polypeptide is capable of specifically binding to CTLA-4 and the IgSF domain of the second polypeptide is capable of binding to a cognate binding partner other than one specifically bound by the CD80 variant polypeptide. In some examples, the IgSF domain of the second polypeptide is a tumor-localizing moiety that binds to a ligand expressed on a tumor. In some examples, the ligand expressed on a tumor is B7H6.

[0048] In some examples, the IgSF domain is from NKp30.

[0049] In some examples, the IgSF domain of the second polypeptide is an IgSF domain of a ligand that binds to an inhibitory receptor, or is an affinity-modified IgSF domain thereof. In some examples, the affinity-modified IgSF domain exhibits increased binding affinity and/or binding selectivity for the inhibitory receptor compared to binding of the unmodified IgSF domain to the inhibitory receptor. In some examples, the inhibitory receptor is TIGIT or PD-1; or the ligand of the inhibitory receptor is CD155, CD112, PD-L1 or PD-L2.

[0050] In some examples, the IgSF domain of the second polypeptide is an affinity-modified IgSF domain containing: (i) a wildtype CD112 comprising an IgSF domain set forth in any of SEQ ID NOS: 269, 734 or 829 or a variant CD112 polypeptide containing an IgSF domain of any of SEQ ID NOS set forth in Table 3, optionally any of the SEQ ID NO: 735-828, 830-917, 918-999, 1430-1501; (ii) a wildtype CD155 comprising an IgSF set forth in any of SEQ ID NOS: 268, 378 or 421 or a variant CD155 polypeptide containing an IgSF domain of any of SEQ ID NOS set forth in Table 4, optionally any of the SEQ ID NO: 379-420, 422-539, 540-733, 1502-1573, 1548-1711, (iii) a wildtype PD-L1 comprising an IgSF set forth in any of SEQ ID NOS: 251, 1000, 1721 or 1196 or a variant PD-L1 polypeptide containing an IgSF of any of SEQ ID NOS set forth in Table 5, optionally any of the SEQ ID NO: 1001-1065, 1718-1900, 1931-1996; (iv) a wildtype PD-L2 comprising an IgSF set forth in any of SEQ ID NOS: 252, 1197 or 1257 variant PD-L2 polypeptide containing an IgSF domain of any of SEQ ID NOS set forth in Table 6, optionally any of the SEQ ID NO: 1198-1248, 1250-1325, 1327-1401, 1403-1426 1719, 1720, 1901-1930, (v) a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 95%, 97%, 98%, 99% or more sequence identity to any of the SEQ ID NOSs in (i)-(iv) and that contains the amino acid substitution; or (vi) a specific binding fragment of any of (i)-(v). In some examples, the IgSF domain is or contains an IgV domain. In some examples, the variant CD80 polypeptide is or contains an IgV domain.

[0051] In some examples, the immunomodulatory protein contains a multimerization domain linked to one or both of the variant CD80 polypeptide and the IgSF domain of the second polypeptide. In some examples, the multimerization domain is an Fc domain or a variant thereof with reduced effector function. In some examples, the immunomodulatory protein is dimeric. In some examples, the immunomodulatory protein is homodimeric. In some examples, the immunomodulatory protein is heterodimeric.

[0052] In some examples, provided herein is a conjugate, containing a variant CD80 polypeptide provided herein or an immunomodulatory polypeptide provided herein linked to a moiety. In some examples, the moiety is a targeting moiety that specifically binds to a molecule on the surface of a cell. In some examples, the targeting moiety specifically binds to a molecule on the surface of an immune cell, optionally wherein the immune cell is an antigen presenting cell or a lymphocyte. In some examples, the immune cell is an antigen presenting cell or a lymphocyte. In some examples, the targeting moiety is a tumor-localizing moiety that binds to a molecule on the surface of a tumor. In some examples, the moiety is a protein, a peptide, nucleic acid, small molecule or nanoparticle. In some examples, the moiety is an antibody or antigen-binding fragment. In some examples, the conjugate is divalent, tetravalent, hexavalent or octavalent. Exemplary depictions of such conjugates are presented in FIG 6A and 6B. In some examples, the conjugate is a fusion protein.

[0053] In some examples, provided herein is a nucleic acid molecule(s), encoding a variant CD80 polypeptide provided herein, an immunomodulatory polypeptide provided herein, or a conjugate that is a fusion protein containing any of the variant CD80 polypeptides provided herein. In some examples, the nucleic acid molecule is synthetic nucleic acid. In some examples, the nucleic acid molecule is cDNA.

[0054] In some examples, provided herein is a vector, containing the nucleic acid molecule provided herein. In some examples, the vector is an expression vector. In some examples, the vector is a mammalian expression vector or a viral vector.

[0055] In some examples, provided herein is a cell, containing a vector provided herein. In some examples, the cell is a mammalian cell. In some examples, the cell is a human cell.

[0056] In some examples, provided herein is a method of producing a variant CD80 polypeptide or an immunomodulatory protein, containing introducing the nucleic acid molecule provided herein or vector provided herein into a host cell under conditions to express the protein

in the cell. In some examples, the method further includes isolating or purifying the variant CD80 polypeptide or immunomodulatory protein from the cell. In one aspect, the present disclosure refers to a method of producing any of the purified CD80-Fc fusion protein disclosed herein or the purified immunomodulatory protein disclosed herein, 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.

[0057] In some examples, provided herein is a method of engineering a cell expressing a variant CD80 variant polypeptide that includes introducing a nucleic acid molecule encoding the variant CD80 polypeptide provided herein into a host cell under conditions in which the polypeptide is expressed in the cell.

[0058] In some examples, provided herein is an engineered cell, expressing a variant CD80 polypeptide provided herein, an immunomodulatory protein provided herein, a conjugate provided herein, the nucleic acid molecule provided herein or the vector provided herein.

[0059] In some examples, the variant CD80 polypeptide or immunomodulatory protein contains a signal peptide. In some examples, the variant CD80 polypeptide or immunomodulatory protein does not contain a transmembrane domain and/or is not expressed on the surface of the cell. In some examples, the variant CD80 polypeptide or immunomodulatory protein is secreted from the engineered cell.

[0060] In some examples, the engineered cell contains a variant CD80 polypeptide that contains a transmembrane domain and/or is a transmembrane immunomodulatory protein provided herein. In some examples, the variant CD80 polypeptide is expressed on the surface of the cell. In some examples, the engineered cell is an immune cell. In some examples, the immune cell is an antigen presenting cell (APC) or a lymphocyte.

[0061] In some examples, the engineered cell is a primary cell. In some examples, the cell is a mammalian cell. In some examples, the cell is a human cell. In some examples, the cell is a lymphocyte that is a T cell. In some examples, the cell is an APC that is an artificial APC. In some examples, the engineered cell further contains a chimeric antigen receptor (CAR) or an engineered T-cell receptor.

[0062] In some examples, provided herein is an infectious agent, containing a nucleic acid molecule encoding a variant CD80 polypeptide provided herein, an immunomodulatory polypeptide provided herein, or a variant CD80 fusion conjugate provided herein. In some examples, the encoded variant CD80 polypeptide or immunomodulatory polypeptide does not contain a transmembrane domain and/or is not expressed on the surface of a cell in which it is expressed. In some examples, the encoded variant CD80 polypeptide, immunomodulatory polypeptide, or conjugate is secreted from a cell in which it is expressed.

[0063] In some examples, the encoded variant CD80 polypeptide contained within the infectious agent contains a transmembrane domain. In some examples, the encoded variant CD80 polypeptide is expressed on the surface of a cell in which it is expressed.

[0064] In some examples, the infectious agent is a bacterium or a virus. In some examples, the virus is a lentiviral or retroviral construct or a hybrid thereof. In some examples, the virus is an oncolytic virus. In some examples, the oncolytic virus is an adenovirus, adeno-associated virus, herpes virus, Herpes Simplex Virus, Reovirus, Newcastle Disease virus, parvovirus, measles virus, vesicular stomatitis virus (VSV), Cocksackie virus or a Vaccinia virus.

[0065] In some examples, the infectious agent is a virus that specifically targets dendritic cells (DCs) and/or is dendritic cell-tropic. In some examples, the virus is a lentiviral vector that is pseudotyped with a modified Sindbis virus envelope product.

[0066] In some examples, the infectious agent further contains a nucleic acid molecule encoding a further gene product that results in death of a target cell or that can augment or boost an immune response. In some examples, the further gene product is selected from an anticancer agent, an anti-metastatic agent, an antiangiogenic agent, an immunomodulatory molecule, an immune checkpoint inhibitor, an antibody, a cytokine, a growth factor, an antigen, a cytotoxic gene product, a pro-apoptotic gene product, an anti-apoptotic gene product, a cell matrix degradative gene, genes for tissue regeneration and reprogramming human somatic cells to pluripotency.

[0067] In some examples, provided herein is a pharmaceutical composition, containing a variant CD80 polypeptide provided herein, an immunomodulatory protein provided herein, a conjugate provided herein, an engineered cell provided herein, or an infectious agent provided herein. In some examples, the pharmaceutical composition contains a pharmaceutically acceptable excipient. In one aspect, the present disclosure refers to a pharmaceutical composition, comprising any of the CD80-Fc fusion protein disclosed herein or the immunomodulatory protein disclosed herein, and a pharmaceutically acceptable excipient. In some examples, the pharmaceutical composition is sterile.

[0068] In some examples, provided herein is an article of manufacture containing the pharmaceutical composition provided herein in a vial. In some examples, the vial is sealed.

[0069] In some examples, provided herein is a kit containing a pharmaceutical composition provided herein, and instructions for use. In some examples, provided herein is a kit containing an article of manufacture provided herein, and instructions for use.

[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, 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,

34, 35, 46, 51, 55, 57, 58, 65, 71, 73, 78, 79, 82, or 84 with reference to numbering of SEQ ID NO: 2.

[0075] In some examples, the one or more modifications are selected from A12T, A12V, T13R, C16R, H18Y, V20I, V22I, V22L, E23D, E23G, E24D, L25S, A26E, A26P, A26S, A26T, Q27H, Q27L, 30F, I30T, I30V, Y31H, Q33E, K34E, E35D, E35G, K36R, M38V, T41A, T41S, M42I, M42V, M43I, M43L, S44P, D46E, D46V, M47I, M47L, M47T, M47V, N48H, P51A, K54E, N55D, N55I, T57A, T57I, I58V, I61V, T62N, L65P, I67L, V68A, V68L, V68M, I69F, I69T, L70M, L70P, L70Q, L70R, A71D, A71G, L72P, L72V, R73S, D76H, E77G, G78A, T79I, T79P, E81K, C82R, V83I, V84A, V84I, L85M, L85Q, K86M, E88D, E88V, D90N, A91S, A91T, F92S, K93R, R94W, E95K and E95V. In some examples, the variant CD80 polypeptide contains one or more amino acid substitutions in an unmodified CD80 or specific binding fragment thereof, selected from among E23D, E23G, A26E, A26P, A26S, A26T, I30F, I30T, I30V, K34E, E35D, E35G, D46E, D46V, P51A, N55D, N55I, T57A, T57I, I58V, L65P, A71D, A71G, R73S, G78A, T79I, T79P, C82R, V84A, V84I, wherein the position(s) of the amino acid substitution(s) correspond(s) to the numbering of positions of CD80 set forth in SEQ ID NO: 2

[0076] In some examples, the one or more modifications are selected from L70P, I30F/L70P, 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/L70P/E77G, T57A/I69T, 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/A91S, 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, V22L/E35G/A71D/L72P, E35D/A71D, E35D/I67L/A71D, Q27H/E35G/A71D/L72P/T79I, T13R/M42V/M47I/A71D, E35D, E35D/M47I/L70M, E35D/A71D/L72V, E35D/M43L/L70M, A26P/E35D/M43I/L85Q/E88D, E35D/D46V/L85Q, Q27L/E35D/M47I/T57I/L70Q/E88D, M47V/I69F/A71D/V83I, E35D/T57A/A71D/L85Q, 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, H18Y/A71D/L72P/E88V, V20I/A71D, E23G/A26S/E35D/T62N/A71D/L72V/L85M,

A12T/E24D/E35D/D46V/I61V/L72P/E95V, V22L/E35D/M43L/A71G/D76H, E35G/K54E/A71D/L72P, L70Q/A71D, A26E/E35D/M47L/L85Q, D46E/A71D, and Y31H/E35D/T41S/V68L/K93R/R94W.

[0077] In some examples, the method includes administering to the subject a soluble variant CD80 polypeptide according to any one of the examples described herein, an immunomodulatory protein according to any one of the examples described or a conjugate according to any one of the examples described herein. In some examples, the method includes administering to the subject an infectious agent encoding a variant CD80 polypeptide according to any one of the examples described herein.

[0078] In some examples, modulating the immune response treats a disease or condition in the subject. In some examples, the immune response is increased. Various formats of a variant CD80 polypeptide are contemplated for administration to a subject to increase an immune response, such as antagonist formats of a variant CD80. In some cases, such methods are carried out under conditions in which signaling by the inhibitory receptor CTLA-4 is blocked or attenuated by the administration.

[0079] In some examples, in the provided methods of modulating an immune response, a variant CD80 polypeptide or immunomodulatory protein that is soluble is administered to the subject. In some examples, the soluble immunomodulatory protein is an immunomodulatory Fc fusion protein.

[0080] In some examples, the provided methods include administering a variant CD80 polypeptide provided herein, or an immunomodulatory protein provided herein, to the subject. In some examples, an engineered cell containing a secretable variant CD80 polypeptide provided herein is administered to the subject. In some examples, an engineered cell provided herein is administered to the subject.

[0081] In some examples of the provided methods, an infectious agent encoding a variant CD80 polypeptide that is a secretable immunomodulatory protein is administered to the subject, optionally under conditions in which the infectious agent infects a tumor cell or immune cell and the secretable immunomodulatory protein is secreted from the infected cell.

[0082] In some examples of the provided methods, the disease or condition is a tumor or cancer. In one aspect, the present disclosure refers to use of the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a tumor or cancer in a subject in need thereof. In some examples, the disease or condition is selected from melanoma, lung cancer, bladder cancer, a hematological malignancy, liver cancer, brain cancer, renal cancer, breast cancer, pancreatic cancer, colorectal cancer, spleen cancer, prostate cancer, testicular cancer,

ovarian cancer, uterine cancer, gastric carcinoma, a musculoskeletal cancer, a head and neck cancer, a gastrointestinal cancer, a germ cell cancer, or an endocrine and neuroendocrine cancer. In some of any such examples, the variant CD80 is administered in a format that increases an immune response in the subject.

[0083] Various formats of a variant CD80 polypeptide are contemplated for administration to a subject to decrease an immune response, such as agonist formats of a variant CD80. In some cases, such methods are carried out under conditions in which signaling by the inhibitory receptor CTLA-4 is activated or stimulated or induced by the administration.

[0084] In some examples of the provided methods, the immune response is decreased. In some examples of the provided methods, an immunomodulatory protein or conjugate containing a variant CD80 polypeptide linked to a moiety that localizes to a cell or tissue of an inflammatory environment is administered to the subject. In some examples, the binding molecule contains an antibody or an antigen-binding fragment thereof or contains a second polypeptide containing a wild-type IgSF domain or variant thereof.

[0085] In some examples of the provided methods, an immunomodulatory protein provided herein or a conjugate provided herein is administered to the subject. In some examples of the provided methods, a variant CD80 polypeptide that is a transmembrane immunomodulatory protein is administered to the subject. In some examples of the provided methods, an engineered cell containing a variant CD80 polypeptide that is a transmembrane immunomodulatory protein provided herein is administered to the subject.

[0086] In some examples of the provided methods, an infectious agent encoding a variant CD80 polypeptide that is a transmembrane immunomodulatory protein is administered to the subject, optionally under conditions in which the infectious agent infects a tumor cell or immune cell and the transmembrane immunomodulatory protein is expressed on the surface of the infected cell.

[0087] In some examples of the provided methods, the disease or condition is an inflammatory or autoimmune disease or condition. In some examples, the disease or condition is an Antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, a vasculitis, an autoimmune skin disease, transplantation, a Rheumatic disease, an inflammatory gastrointestinal disease, an inflammatory eye disease, an inflammatory neurological disease, an inflammatory pulmonary disease, an inflammatory endocrine disease, or an autoimmune hematological disease. In some examples, the disease or condition is selected from

inflammatory bowel disease, transplant, Crohn's disease, ulcerative colitis, multiple sclerosis, asthma, rheumatoid arthritis, or psoriasis. In some of any such examples, the variant CD80 is administered in a format that decreases an immune response in the subject.

[0088] In some examples, provided herein is a method of treating a disease or condition including administering an immunomodulatory protein, containing a variant CD80 polypeptide, containing one or more amino acid modifications at one or more position sin the IgV domain or IgC domain or a specific binding fragment thereof of in an unmodified CD80 or specific binding fragment thereof, wherein the immunomodulatory protein exhibits PD-L1 dependent CD28 costimulation. In some examples, the variant CD80 polypeptide exhibits increased binding affinity to the ectodomain of PD-L1 compared to the binding affinity of the unmodified CD80 for the ectodomain of PD-L1. In some examples, provided herein is a method of mediating CD28 agonism by PD-L1-dependent CD28 costimulation in a subject, the method comprising administering an immunomodulatory protein comprising a variant CD80 polypeptide, said variant CD80 polypeptide comprising one or more amino acid modifications at one or more positions in the IgV domain or IgC domain or the specific binding fragment thereof of an unmodified CD80 or specific binding fragment thereof, wherein the variant CD80 polypeptide exhibits increased binding affinity to the ectodomain of PD-L1 compared to the binding affinity of the unmodified CD80 for the ectodomain of PD-L1. In any of the examples, the increased affinity to the ectodomain of PD-L1 is 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 compared to binding affinity of the unmodified CD80 for the ectodomain of PD-L1.

[0089] In some examples the method is for use in treating a disease or condition. In some examples, PD-L1-dependent CD28 costimulation is assessed in a T cell stimulation assay in the presence of antigen presenting cells expressing PD-L1, optionally wherein the T cell stimulation assay is an in vitro assay, optionally wherein the T cells comprise Jurkat cells expressing an IL-2 reporter or primary human T cells producing inflammatory cytokines such as IL-2.

[0090] In some examples of the provided methods, prior to the administering, a subject for treatment is selected that has a tumor comprising cells positive for surface PD-L1, optionally wherein the cells are tumor cells or tumor infiltrating immune cells; or the subject has been selected as having a tumor comprising cells surface positive for PD-L1, optionally wherein the cells are tumor cells or tumor infiltrating immune cells.

[0091] In some examples, selecting a subject comprises (a) contacting a tumor tissue sample from a subject with a binding reagent capable of specifically binding the ectodomain of PD-L1; (b) detecting the presence of the bound binding reagent in or on cells of the tumor tissue sample, optionally wherein the cells are tumor cells or tumor infiltrating immune cells; and (c) if the tumor tissue sample comprises a detectable level of cells surface positive for PD-L1, selecting the subject for treatment.

[0092] In some examples of the provided methods, prior to the administering, a subject for treatment is selected that has a tumor comprising cells surface positive for CD28, optionally wherein the cells are tumor infiltrating lymphocytes, optionally wherein the lymphocytes are T cells, optionally CD8+ T cells; or the subject has been selected as having a tumor comprising cells surface positive for CD28, optionally wherein the cells are tumor infiltrating lymphocytes, optionally wherein the lymphocytes are T cells, optionally CD8+ T cells.

[0093] In some examples, selecting the subject includes (a) contacting a tumor tissue sample from a subject with a binding reagent capable of specifically binding the ectodomain of CD28; (b) detecting the presence of the bound binding reagent in or on cells of the tumor tissue sample, optionally wherein the cells are tumor infiltrating lymphocytes, optionally wherein the lymphocytes are T cells, optionally CD8+ T cells; and (c) if the tumor tissue sample comprises a detectable level of cells surface positive for CD28, selecting the subject for treatment.

[0094] In some examples, provided herein are methods of selecting a subject for treatment, the methods including: (a) contacting a tumor tissue sample from a subject with a binding reagent capable of specifically binding PD-L1; and (b) detecting the presence of the bound binding reagent in or on cells of the tumor tissue sample, optionally wherein the cells are tumor cells or tumor infiltrating immune cells; and (c) if the tumor sample comprises a detectable level of cells surface positive for PD-L1, selecting the subject for treatment with an immunomodulatory protein comprising a variant CD80 polypeptide, said variant CD80 polypeptide comprising one or more amino acid modifications at one or more positions in the IgV domain or IgC domain or the specific binding fragment thereof of an unmodified CD80 or specific binding fragment thereof, wherein the variant CD80 polypeptide exhibits increased binding affinity to the ectodomain of PD-L1 compared to the binding affinity of the unmodified CD80 for the ectodomain of PD-L1.

[0095] In some examples, the methods further include contacting the tumor tissue sample with a binding reagent capable of specifically binding CD28, wherein the subject is selected if

the tumor tissue sample further comprises a detectable level of tumor infiltrating lymphocytes positive for CD28, optionally wherein the lymphocytes are T cells, optionally CD8+ T cells.

[0096] In some examples, the tumor tissue sample contains tumor infiltrating immune cells, tumor cells, stromal cells, or any combination thereof.

[0097] In some examples, the binding reagent is an antibody or antigen-binding fragment, protein ligand or binding partner, an aptamer, an affimer, a peptide or a hapten. In some examples, the binding reagent is an anti-PD-L1 antibody or antigen-binding fragment. In some examples, the binding reagent is a variant CD80 polypeptide provided herein. In some examples, the variant CD80 polypeptide comprises the IgV domain or a specific binding fragment thereof. In some examples, the IgV domain or specific binding fragment thereof is the only CD80 portion of the binding reagent.

[0098] In some examples, the variant CD80 polypeptide exhibits increased affinity for binding to PD-L1 compared to the wildtype or unmodified CD80 polypeptide.

[0099] In some examples, the binding reagent is linked, directly or indirectly, to a moiety that is a detectable moiety or a moiety capable of detection. In some examples, the moiety is an Fc region. In some examples, the Fc region is non-human, optionally is mouse or rabbit.

[0100] In some examples, detecting the presence of bound binding reagent is by immunohistochemistry, pseudo-immunohistochemistry, immunofluorescence, flow cytometry, ELISA or immunoblotting.

[0101] In some examples, the methods further include administering the immunomodulatory protein to the subject. In some examples, the subject is a human subject.

[0102] In some examples, the immunomodulatory protein is a multimer comprising a first variant CD80 polypeptide linked to a first multimerization domain and a second variant CD80 polypeptide linked to a second multimerization domain, wherein the first and second multimerization domain interact to form a multimer comprising the first and second variant CD80 polypeptide. In some examples, the multimer is a dimer. In some examples, the first variant CD80 polypeptide and the second variant CD80 polypeptide are the same.

[0103] In some examples, the multimerization domain is or comprises an Fc region, optionally a variant Fc region containing one or more amino acid substitutions compared to a wildtype Fc region, wherein the Fc region exhibits one or more effector function that is reduced compared to the wildtype Fc region, optionally wherein the wildtype Fc is human IgG1.

[0104] In some of such examples, the CD80 polypeptide contains one or more amino acid modifications in an unmodified CD80 or specific binding fragment thereof, corresponding to position(s) 7, 12, 13, 15, 16, 18, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 41, 42, 43, 44, 46, 47, 48, 51, 53, 54, 55, 57, 58, 61, 62, 63, 65, 67, 68, 69, 70, 71, 72, 73, 74, 76, 77, 78, 79, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, and/or 97, with reference to numbering of SEQ ID NO: 2. In some of such examples, the CD80 polypeptide contains one or more amino acid modifications in an unmodified CD80 or specific binding fragment thereof, selected from among E7D, A12V, T13A, T13R, S15P, C16R, H18L, H18Y, V20A, V20I, V22A, V22D, V22I, V22L, E23D, E23G, E24D, L25S, A26E, A26P, A26S, A26T, Q27H, T28Y, R29H, I30T, I30V, Y31H, Y31S, Q33E, Q33H, Q33K, Q33L, Q33R, K34E, E35D, K36R, K37E, M38T, M38V, T41A, T41S, M42I, M42V, M43I, M43L, M43T, M43V, S44P, D46E, D46V, M47I, M47L, M47T, M47V, N48D, N48H, N48K, N48R, N48S, N48T, P51A, Y53F, Y53H, K54R, N55D, N55I, T57I, I58V, I61N, I61V, T62A, T62N, T62S, N63D, L65P, I67L, I67T, V68A, V68L, V68M, I69F, L70M, L70P, L70Q, L70R, A71D, A71G, L72P, L72V, R73S, P74S, D76H, E77A, G78A, T79A, T79I, T79L, T79P, E81G, E81K, C82R, V83A, V83I, V84A, V84I, L85E, L85M, L85Q, K86E, K86M, Y87H, Y87N, Y87Q, E88D, E88G, K89E, K89N, D90G, D90N, A91T, A91V, F92L, F92S, F92V, F92Y, K93E, K93R, K93T, R94Q, R94W, E95D, E95K, E95V, L97M, L97Q, and L97R, wherein the position(s) of the amino acid substitution(s) correspond(s) to the positions of CD80 set forth in SEQ ID NO: 2.

[0105] In some examples, the variant CD80 polypeptide retains binding to CD28. In some examples, the variant CD80 polypeptide retains at least or at least about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the affinity to the ectodomain of CD28, compared to the binding affinity of the unmodified CD80 polypeptide for the ectodomain of CD28. In some examples, the variant CD80 polypeptide exhibits increased binding affinity to the ectodomain of CD28 compared to the binding affinity of the unmodified CD80 for the ectodomain of CD28. In some of such examples, the increased affinity to the ectodomain of CD28 is 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 compared to binding affinity of the unmodified CD80 for the ectodomain of CD28.

[0106] In some examples, increasing the immune response treats a disease or condition in the subject. In some examples the disease or condition is a tumor or cancer. In some

examples, the disease or condition is selected from melanoma, lung cancer, bladder cancer, a hematological malignancy, liver cancer, brain cancer, renal cancer, breast cancer, pancreatic cancer, colorectal cancer, spleen cancer, prostate cancer, testicular cancer, ovarian cancer, uterine cancer, gastric carcinoma, a musculoskeletal cancer, a head and neck cancer, a gastrointestinal cancer, a germ cell cancer, or an endocrine and neuroendocrine cancer.

[0107] In some examples, provided herein is a method of detecting a CD80 binding partner in a biological sample, the method comprising: (a) contacting a biological sample with a binding reagent comprising any of the variant CD80 polypeptides provided herein; and (b) detecting the presence of the bound binding reagent in or on cells of the biological sample. In some examples, the binding partner is PD-L1, CD28, CTLA-4 or combinations thereof.

[0108] In some examples, the variant CD80 polypeptide comprises one or more amino acid modifications at one or more positions in the IgV domain or IgC domain or the specific binding fragment thereof of an unmodified CD80 or specific binding fragment thereof, wherein the variant CD80 polypeptide exhibits increased binding affinity to the ectodomain of PD-L1 compared to the binding affinity of the unmodified CD80 for the ectodomain of PD-L1.

[0109] In some examples, the biological sample is or comprises a body fluid, cell or tissue sample, such as body fluid that is serum, plasma or urine or a tissue sample that is a tumor tissue sample. In some examples, the tumor tissue sample contains tumor infiltrating immune cells, tumor cells, stromal cells, or any combination thereof.

[0110] In some examples, the variant CD80 polypeptide comprises the IgV domain or a specific binding fragment thereof. In some examples, the IgV domain or specific binding fragment thereof is the only CD80 portion of the binding reagent.

[0111] In some examples, the binding reagent is linked, directly or indirectly, to a label that is a detectable moiety or to a moiety capable of detection. In some examples, the moiety is an Fc region, that is optionally non-human, such as a mouse or rabbit Fc region. In some examples, detecting the presence of bound binding reagent is by immunohistochemistry, pseudo-immunohistochemistry, immunofluorescence, flow cytometry, ELISA or immunoblotting.

BRIEF DESCRIPTION OF THE DRAWINGS

[0112] FIG. 1A-1C depicts various formats of the provided variant IgSF domain molecules. **FIG. 1A** depicts soluble molecules, including: (1) a variant IgSF domain (vIgD) fused to an Fc chain; (2) a stack molecule containing a first variant IgSF domain (first vIgD) and a second IgSF

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 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 exhibits increased binding affinity to the ectodomain of human PD-L1 compared to the binding affinity of the unmodified 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 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 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 claim 4, wherein the increase 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 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/D90G,
 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: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.

44. 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.