

been used to treat cancer and induce immune responses. The tumor microenvironment is a hostile environment surrounding tumors, which is highly immunosuppressive and a major barrier for cancer therapies to eliminate solid tumors effectively. Immunosuppressive factors, like PD-L1 and TGF β , produced by the tumor or stromal cells and resident in the tumor microenvironment, suppress the activity of immune cells, thereby limiting the ability of the immune system to act against the invading cancer. Therefore, autologous immune cell therapies are not sufficient for efficiently treating cancers, especially solid cancers.

[0003] There is a need for more effective and safer classes of cell therapies that could treat cancer and induce immune responses *in vivo*, that also overcome the immunological obstacles of the tumor microenvironment. The present disclosure addresses this unmet need.

SUMMARY

[0004] The present disclosure relates to engineered proteins (e.g., chimeric proteins) that are capable, when present on a cell, of inhibiting immunosuppressive signals that exist in the tumor microenvironment. The present disclosure further relates to engineered cells, e.g., immune cells such as natural killer (NK) cells, comprising one or more of said engineered proteins (e.g., chimeric proteins), as well as to methods of using the engineered cells for treating a disease or disorder, such as cancer. The engineered proteins (e.g., chimeric proteins) comprise an extracellular domain, a transmembrane domain, and optionally an intracellular domain, and in some [embodiment examples](#), are chimeric proteins. These proteins, when present on a cell, such as an NK cell, are capable of inhibiting immunosuppressive signals by binding to the negative signaling molecule and acting as a sink or as a dominant negative receptor, thereby neutralizing the negative signal, or acting as a signal inverter to convert the negative signal that would have otherwise been inhibitory into an activating signal, thereby enhancing the anti-tumor activity of the cell.

[0005] The activation of NK cells relies more heavily on, and on a broader repertoire of, signaling-dependent receptors, such as DAP10 and DAP12, in comparison to other types of leukocytes, such as T cells, B cells, monocytes, and macrophages. Therefore, signal inverters (e.g., TGF- β R/DAP10 or TGF- β R/DAP12) that convert negative signals associated with the cell's immunosuppressive activity (e.g., by TGF- β R) to a positive signal, offer a selective advantage to NK cells over other immune cell types. Furthermore, while T cell activation and

behavior is highly dependent on TCR engagement, NK cell activation and subsequent target cell killing is, in contrast, determined by a balance of activating and inhibitory signals, and is not as dependent on a single signal. Therefore, while not wishing to be bound by theory, it is believed that incorporating an additional activating signal into NK cells could facilitate a meaningful change in that balance and substantially alter NK cell behavior. It is further postulated that the activating signals provided by, for example, a TGF- β R signal inverter could facilitate intrinsic gains in function of the cell (e.g., NK cell), by preventing antigen escape, by enhancing the expression of endogenous NK activating receptors and/or by promoting a favorable phenotype for anti-tumor activity (e.g., differentiation state) by improving metabolic fitness within the tumor microenvironment. The activating signals provided by, for example, a TGF- β R signal inverter may also facilitate extrinsic gains in function of the cell (e.g., NK cell) by disrupting immunosuppression in the tumor microenvironment, for example, by improving inflammation mediated by chemokines or cytokines, and/or by driving immune activation and epitope spreading with costimulatory ligands or cytokines.

[0006] Also provided herein are chimeric proteins that include an extracellular domain, a transmembrane domain, and an intracellular domain, wherein the extracellular domain is capable of binding a negative signal, and wherein the intracellular domain comprises an intracellular domain, or a portion thereof, of a stimulatory polypeptide.

[0006a] In one aspect, the present disclosure refers to a chimeric protein comprising an extracellular domain, a transmembrane domain, and an intracellular domain, wherein the extracellular domain is capable of binding a negative signal, and wherein the intracellular domain comprises an intracellular domain, or a portion thereof, of a stimulatory polypeptide, and the negative signal is selected from the group consisting of TGF- β , IL-10, IL-1, IL-6, PD-L1, PD-L2, B7-1, B7-2, MHCI, HVEM, CD155, CD112, CD111, CD200, B7-H6, HS-GAG, HLA, N-cadherin, E-cadherin, FasL, MHCII, TIM-3, IL-18, adenosine, and prostaglandin, wherein the stimulatory polypeptide is selected from the group consisting of: DAP10, DAP12, 2B4, CD2, IL-21, CARD11, LAT, LAT2, SLP76, LAX, MyD88, PAG, GAPT, SAP, EAT-2, FcRK, FcRI, FcKRIIa, FcKRIIc, FcKRIIIa, LFA-3, SLAM, SLAMF5, SLAMF6, SLAMF7, SLAMF3, CEACAM-3, CD209, CLEC-2, CLEC-7A, CD370, NKp80, NKp65, NKG2C, NKG2D, NKG2E, CD131, TSLP-R, CD116, IL-1R3, IL-1RA, IL-1RL1, IL-1RL2, IL-11RA, IL-17RA, IL-17RB, IL-17RC, IL-17RE, IL-18R1, IL-18RB, IL-22RA1, IL-23R, IL-27RA, IL-3RA, LIF-R, CSF-1R, OSM-RB, CD220, LEP-R, CD79A, CD79B, DNAM-1, CD83, ICOS, ICAM-1, ICAM-2, ICAM-3, KIR2DL4, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, NKp46, NKp44, NKp30, CD7, CD4, CD28, TMIGD2, CD11A, CD18, CD355, CD22, CD3E, CD3G, CD3Z, Lck, ZAP70, TIM-1, TLR1, TLR10, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, CD27, CD70, CD258, CD153, RANK, CD266, CD267, CD268, CD270, CD271, CD269, CD357, TNFRSF19, TNFRSF19L, CD120A, CD120B, TNFRSF25, TNFRSF27, TNFRSF3, OX-40, CD40, CD30, 4-1BB, EDAR, PILRB, FCARI, CD58, CD35, CD46, CD206, HER2, HGFR, CD331, CD332, TNFSF18, CD257, CD256, TWEAK, CD254, CD253, CD333, CD334, CD309, VEGFR-3, EPHA1, EPHB1, CD140a, CD140b, CD160, ICAM-5, SIGLEC-14, SIGLEC-15, SIGLEC-16, TROY, RELT, TRAMP, XEDAR, LTB-R, CD23, CLEC-4E, CLEC-6A, CLEC-10A, CLEC-4D, CLEC-4C, TNFSF1A, OX40L, CD40L, CD178, and 4-1BBL.

[0007] In some embodimentexamples, the chimeric protein is capable of activating an immune cell selected from an NK cell, an NKT cell, a T-cell, and a macrophage.

[0008] In some embodimentexamples, the chimeric protein is capable of activating an NK cell.

[0009] In some embodimentexamples, the extracellular domain comprises an antigen-binding domain that specifically binds to the negative signal. In some embodimentexamples, the antigen-binding domain comprises a fragment of an antibody. In some embodimentexamples, the antigen-binding domain comprises an scFv, a Fab, or a VHH. In some embodimentexamples, the scFv is an scFv from a monoclonal antibody. In some embodimentexamples, the scFv is connected to the transmembrane domain by a linker.

[0010] In some embodimentexamples, the antigen-binding domain specifically binds to a negative signal selected from the group consisting of TGF- β , IL-10, IL-1, IL-6, PD-L1, PD-L2, B7-1, B7-2, MHCII, HVEM, CD155, CD112, CD111, CD200, B7-H6, HS-GAG, HLA, N-cadherin, E-cadherin, FasL, and MHCII.

[0011] In some [embodimentexamples](#), the extracellular domain comprises the extracellular domain, or a portion thereof, of an inhibitory polypeptide that binds the negative signal. In some [embodimentexamples](#), the inhibitory polypeptide is an inflammatory mediator receptor, an inhibitory cytokine receptor, an immune checkpoint receptor, or a dual activator-checkpoint receptor.

[0012] In some [embodimentexamples](#), the inhibitory polypeptide is selected from the inhibitory polypeptides presented in Table 1 or Table 1.1.

[0013] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of an inhibitory polypeptide presented in Table 1 or Table 1.2.

[0014] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of a stimulatory polypeptide presented in Table 2 or Table 2.2.

[0015] In some [embodimentexamples](#), the intracellular domain comprises the intracellular domain, or a portion thereof, of a stimulatory polypeptide selected from the stimulatory polypeptides presented in Table 2 or Table 2.1. In some [embodimentexamples](#), the stimulatory polypeptide is selected from one or more isoforms of the stimulatory polypeptide.

[0016] In some [embodimentexamples](#), the chimeric protein comprises the intracellular domain, or a portion thereof, of two or more different stimulatory polypeptides.

[0017] In some [embodimentexamples](#), each of the extracellular domain, the transmembrane domain, and the intracellular domain have the same N-terminal to C-terminal orientation.

[0018] In some [embodimentexamples](#), the inhibitory polypeptide is a type I receptor, and the stimulatory polypeptide is a type I receptor; the inhibitory polypeptide is a type I receptor, and the stimulatory polypeptide is a type III receptor; the inhibitory polypeptide is a type II receptor, and the stimulatory polypeptide is a type II receptor; the inhibitory polypeptide is a type I receptor, and the stimulatory polypeptide is not associated with the plasma membrane; or the inhibitory polypeptide is a type I receptor, the stimulatory polypeptide is a type II receptor, and the transmembrane domain, or portion thereof, is from a type I receptor.

[0019] In some [embodimentexample](#), the inhibitory polypeptide is capable of forming a dimer and the stimulatory polypeptide is capable of forming a dimer; or wherein the inhibitory polypeptide is capable of forming a trimer and the stimulatory polypeptide is capable of forming a trimer.

[0020] In some [embodimentexamples](#), a combination of the extracellular domain, or a portion thereof, of an inhibitory polypeptide and the intracellular domain, or a portion thereof, of a stimulatory polypeptide is selected from the combinations presented in any one of Tables 6-14.

[0021] In some [embodimentexamples](#), the extracellular domain and the transmembrane domain are connected by a linker. In some [embodimentexamples](#), the linker is selected from the linkers presented in Table 3.

[0022] In some [embodimentexamples](#), the transmembrane domain and the intracellular domain are connected by a linker. In some [embodimentexamples](#), the linker is selected from the linkers presented in Table 3.

[0023] Also provided herein are modified immune cells engineered to express a chimeric protein comprising an extracellular domain, a transmembrane domain, and an intracellular domain, wherein the extracellular domain is capable of binding a negative signal, and wherein the intracellular domain comprises an intracellular domain, or a portion thereof, of a stimulatory polypeptide. In one aspect, the present disclosure refers to a modified immune cell engineered to express a chimeric protein as disclosed herein.

[0024] In some [embodimentexamples](#), the immune cell is selected from the group consisting of an NK cell, an NKT cell, a T-cell, and a macrophage. In some [embodimentexamples](#), the immune cell is an NK cell.

[0025] In some [embodimentexamples](#), the extracellular domain comprises an antigen-binding domain that specifically binds to the negative signal. In some [embodimentexamples](#), the antigen-binding domain comprises a fragment of an antibody. In some [embodimentexamples](#), the antigen-binding domain comprises an scFv, a Fab, or a VHH. In some [embodimentexamples](#), the scFv is an scFv from a monoclonal antibody. In some [embodimentexamples](#), the scFv is connected to the transmembrane domain by a linker.

[0026] In some [embodimentexamples](#), the antigen-binding domain specifically binds to a negative signal selected from the group consisting of TGF- β , IL-10, IL-1, IL-6, PD-L1, PD-L2, B7-1, B7-2, MHCII, HVEM, CD155, CD112, CD111, CD200, B7-H6, HS-GAG, HLA, N-cadherin, E-cadherin, FasL, and MHCII.

[0027] In some [embodimentexamples](#), the extracellular domain comprises the extracellular domain, or a portion thereof, of an inhibitory polypeptide. In some [embodimentexamples](#), the inhibitory polypeptide is an inflammatory mediator receptor, an inhibitory cytokine receptor, an immune checkpoint receptor, or a dual activator-checkpoint receptor. In some [embodimentexamples](#), the inhibitory polypeptide is selected from the inhibitory polypeptides presented in Table 1 or Table 1.1.

[0028] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of an inhibitory polypeptide presented in Table 1 or Table 1.2.

[0029] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of a stimulatory polypeptide presented in Table 2 or Table 2.2.

[0030] In some [embodimentexamples](#), the intracellular domain comprises the intracellular domain, or a portion thereof, of a stimulatory polypeptide selected from the stimulatory polypeptides presented in Table 2 or Table 2.1.

[0031] In some [embodimentexamples](#), the chimeric protein comprises the intracellular domain, or a portion thereof, of two or more different stimulatory polypeptides.

[0032] In some [embodimentexamples](#), the extracellular domain and the transmembrane domain are connected with a linker. In some [embodimentexamples](#), the linker is selected from the linkers presented in Table 3.

[0033] In some [embodimentexamples](#), the transmembrane domain and the intracellular domain are connected with a linker. In some [embodimentexamples](#), the linker is selected from the linkers presented in Table 3.

[0034] In some [embodimentexamples](#), the immune cell is engineered to further comprise a chimeric antigen receptor (CAR). In some [embodimentexamples](#), the CAR targets a tumor antigen.

[0035] In some [embodimentexamples](#), the immune cell is engineered to further comprise a cytokine. In some [embodimentexamples](#), the cytokine can be selected from the group consisting of a chemokine, an interferon, an interleukin, a lymphokine, a tumor necrosis factor, or a variant or combination thereof. In some [embodimentexamples](#), the cytokine is an IL-15 or a fragment or variant thereof.

[0036] Also provided herein are chimeric proteins that include an extracellular domain, a transmembrane domain, and an intracellular domain, wherein the extracellular domain binds to TGF- β , and wherein the intracellular domain comprises an intracellular domain, or a portion thereof, of a stimulatory polypeptide.

[0037] In some [embodimentexamples](#), the chimeric protein is capable of activating an immune cell selected from the group consisting of an NK cell, an NKT cell, a T-cell, and a macrophage.

[0038] In some [embodimentexamples](#), the chimeric protein is capable of activating an NK cell.

[0039] In some [embodimentexamples](#), the extracellular domain comprises an antigen-binding domain that specifically binds TGF- β . In some [embodimentexamples](#), the antigen-binding domain comprises a fragment of an antibody. In some [embodimentexamples](#), the antigen-binding domain comprises an scFv, a Fab, or a VHH. In some [embodimentexamples](#), the scFv is an scFv from a monoclonal antibody. In some [embodimentexamples](#), the scFv is connected to the transmembrane domain by a linker.

[0040] In some [embodimentexamples](#), the extracellular domain comprises the extracellular domain, or a portion thereof, of a TGF- β receptor polypeptide.

[0041] In some [embodimentexamples](#), the extracellular domain comprises the extracellular domain, or a portion thereof, of a TGF- β R1 polypeptide or a TGF- β R2 polypeptide presented in Table 1.1.

[0042] In some [embodimentexamples](#), the intracellular domain comprises the intracellular domain, or a portion thereof, of a stimulatory polypeptide selected from the stimulatory polypeptides presented in Table 2 or Table 2.1.

[0043] In some [embodimentexamples](#), the intracellular domain comprises the intracellular domain, or a portion thereof, of two or more different stimulatory polypeptides.

[0044] In some [embodimentexamples](#), the stimulatory polypeptide is selected from the group consisting of DAP10, DAP12, 2B4, CD2, LFA1, IL-21, and PILRB.

[0045] In some [embodimentexamples](#), the stimulatory polypeptide is not one or more of BMP, IL-1, IL-2, IL-7, IL-15, IL-21, IL-12, IL-18, IL-19, IFN-gamma, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, CD28, 4-1BB, OX40, CD3 (CD3zeta), CD40, CD27, IL-12R, IL-7R, CD137, and ICOS .

[0046] In some [embodimentexamples](#), the stimulatory polypeptide is not DAP12.

[0047] In some [embodimentexamples](#), the extracellular domain further comprises at least a portion of an extracellular domain of the stimulatory polypeptide.

[0048] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of a stimulatory polypeptide presented in Table 2 or Table 2.2.

[0049] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of the TGF- β receptor.

[0050] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of the stimulatory polypeptide.

[0051] In some [embodimentexamples](#), the transmembrane domain and the stimulatory polypeptide are respectively selected from the group consisting of: DAP12 and DAP12; TGF- β R2 and DAP12; 2B4 and 2B4; TGF- β R2 and 2B4; LFA1 and LFA1; TGF- β R2 and LFA1; CD2 and CD2; TGF- β R2 and CD2; CD28 and CD28+CD3zeta; and CD28H and CD28H+CD3zeta.

[0052] In some [embodimentexamples](#), the extracellular domain and transmembrane domain are connected by a linker. In some [embodimentexamples](#), the linker is selected from the linkers presented in Table 3.

[0053] In some [embodimentexamples](#), the transmembrane domain and intracellular domain are connected by a linker. In some [embodimentexamples](#), the linker is selected from the linkers presented in Table 3.

[0054] Also provided herein are modified immune cells engineered to express a chimeric protein comprising an extracellular domain, a transmembrane domain, and an intracellular domain, wherein the extracellular domain binds to TGF- β , and wherein the intracellular domain comprises an intracellular domain, or a portion thereof, of a stimulatory polypeptide.

[0055] In some [embodimentexamples](#), the immune cell is selected from the group consisting of an NK cell, an NKT cell, a T-cell, and a macrophage. In some [embodimentexamples](#), the immune cell is an NK cell.

[0056] In some [embodimentexamples](#), the intracellular domain comprises the intracellular domain, or a portion thereof, of a stimulatory polypeptide that promotes activation of an NK cell.

[0057] In some [embodimentexamples](#), the binding of the extracellular domain to TGF- β activates the immune cell.

[0058] In some [embodimentexamples](#), the extracellular domain comprises an antigen-binding domain that specifically binds TGF- β . In some [embodimentexamples](#), the antigen-binding domain comprises a fragment of an antibody. In some [embodimentexamples](#), the antigen-binding domain comprises an scFv, a Fab, or a VHH. In some [embodimentexamples](#), the scFv is an scFv from a monoclonal antibody.

[0059] In some [embodimentexamples](#), the extracellular domain comprises the extracellular domain, or a portion thereof, of a TGF- β receptor (TGF-BR or TGF- β R) polypeptide.

[0060] In some [embodimentexamples](#), the extracellular domain comprises the extracellular domain, or a portion thereof, of a TGF- β R1 polypeptide or a TGF- β R2 polypeptide presented in Table 1.1.

[0061] In some [embodimentexamples](#), the intracellular domain comprises the intracellular domain, or a portion thereof, of a stimulatory polypeptide selected from the stimulatory polypeptides presented in Table 2 or Table 2.1.

[0062] In some [embodimentexamples](#), the intracellular domain comprises the intracellular domain, or a portion thereof, of two or more different stimulatory polypeptides.

[0063] In some [embodimentexamples](#), the stimulatory polypeptide is DAP10, DAP12, 2B4, CD2, LFA1, IL-21, or PILRB.

[0064] In some [embodimentexamples](#), the stimulatory polypeptide is not one or more of BMP, IL-1, IL-2, IL-7, IL-15, IL-21, IL-12, IL-18, IL-19, IFN-gamma, TLR1, TLR2, TLR3, TLR4, TLR5,

TLR6, TLR7, TLR8, TLR9, CD28, 4-1BB, OX40, CD3 (CD3zeta), CD40, CD27, IL-12R, IL-7R, CD137, and ICOS.

[0065] In some [embodimentexamples](#), the stimulatory polypeptide is not DAP12.

[0066] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of a stimulatory polypeptide presented in Table 2 or Table 2.2.

[0067] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of the TGF- β receptor (TGF-BR).

[0068] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of the stimulatory polypeptide.

[0069] In some [embodimentexamples](#), the transmembrane domain and the stimulatory polypeptide are respectively selected from the group consisting of: DAP12 and DAP12; TGF- β R2 and DAP12; 2B4 and 2B4; TGF- β R2 and 2B4; LFA1 and LFA1; TGF- β R2 and LFA1; CD2 and CD2; TGF- β R2 and CD2; CD28 and CD28+CD3zeta; and CD28H and CD28H+CD3zeta.

[0070] In some [embodimentexamples](#), the immune cell is engineered to further comprise a chimeric antigen receptor (CAR). In some [embodimentexamples](#), the CAR targets a tumor antigen.

[0071] In some [embodimentexamples](#), the immune cell is engineered to further comprise a cytokine. In some [embodimentexamples](#), the cytokine can be selected from the group consisting of a chemokine, an interferon, an interleukin, a lymphokine, a tumor necrosis factor, or a variant or combination thereof. In some [embodimentexamples](#), the cytokine is an IL-15 or a fragment or variant thereof.

[0072] Also provided herein are proteins that include an extracellular domain and a transmembrane domain, wherein the extracellular domain is capable of binding a negative signal, and wherein the protein lacks a fully functional intracellular domain.

[0073] In some [embodimentexamples](#), the protein lacks an intracellular domain.

[0074] In some [embodimentexamples](#), the extracellular domain comprises an antigen-binding domain that specifically binds to the negative signal. In some [embodimentexamples](#), the antigen-binding domain comprises a fragment of an antibody. In some [embodimentexamples](#), the antigen-binding domain comprises an scFv, a Fab, or a VHH. In some [embodimentexamples](#), the scFv is an scFv from a monoclonal antibody. In some [embodimentexamples](#), the scFv is connected to the transmembrane domain by a linker.

[0075] In some [embodimentexamples](#), the extracellular domain comprises the extracellular domain, or a portion thereof, of an inhibitory polypeptide that binds to the negative signal. In some

embodimentexamples, the inhibitory polypeptide is an inflammatory mediator receptor, an inhibitory cytokine receptor, an immune checkpoint receptor, or a dual activator-checkpoint receptor.

[0076] In some embodimentexamples, the inhibitory polypeptide is selected from the inhibitory polypeptides presented in Table 1 or Table 1.1.

[0077] In some embodimentexamples, the transmembrane domain comprises the transmembrane domain, or a portion thereof, of an inhibitory polypeptide presented in Table 1 or Table 1.2.

[0078] In some embodimentexamples, the transmembrane domain comprises the transmembrane domain, or a portion thereof, of a stimulatory polypeptide presented in Table 2 or Table 2.2.

[0079] In some embodimentexamples, the extracellular domain and the transmembrane domain are connected by a linker. In some embodimentexamples, the linker is selected from the linkers presented in Table 3.

[0080] In some embodimentexamples, the transmembrane domain comprises the transmembrane domain, or a portion thereof, of a different polypeptide than the extracellular domain. In some embodimentexamples, the different polypeptide is selected from the inhibitory polypeptides presented in Table 1 or Table 1.2; or the stimulatory polypeptides presented in Table 2 or Table 2.2.

[0081] Also provided herein are modified cells engineered to express a protein comprising an extracellular domain and a transmembrane domain, wherein the extracellular domain is capable of binding a negative signal, and wherein the chimeric protein lacks a fully functional intracellular domain.

[0082] In some embodimentexamples, the cell is selected from the group consisting of an artificial cell, an immune cell, a fibrocyte, a mesenchymal stem cell, an induced neural stem cell, or an induced pluripotent stem cell (iPSC)-derived cell, and an erythrocyte. In some embodimentexamples, the immune cell is selected from the group consisting of a T cell, an NK cell, an NKT cell (e.g., an invariant NKT (iNKT) cell), a type 1 innate lymphoid cell (ILC1), an intraepithelial type 1 innate lymphoid cell (ieILC1), a type 2 innate lymphoid cell (ILC2), a type 3 innate lymphoid cell (ILC3), a lymphoid tissue inducer cell (LTi), a monocyte, a macrophage, a dendritic cell (DC), a platelet, a marrow-infiltrating lymphocyte (MIL), and a B cell. In some embodimentexamples, the immune cell is an NK cell.

[0083] In some embodimentexamples, the chimeric protein lacks an intracellular domain.

[0084] In some embodimentexamples, the extracellular domain comprises an antigen-binding domain that specifically binds to the negative signal. In some embodimentexamples, the antigen-binding domain

comprises a fragment of an antibody. In some [embodimentexamples](#), the antigen-binding domain comprises an scFv, a Fab, or a VHH. In some [embodimentexamples](#), the scFv is an scFv from a monoclonal antibody. In some [embodimentexamples](#), the extracellular domain does not comprise an antigen-binding domain (e.g., an antibody or a fragment thereof, scFv, Fab, and a VHH).

[0085] In some [embodimentexamples](#), the extracellular domain comprises the extracellular domain, or a portion thereof, of an inhibitory polypeptide that binds to the negative signal. In some [embodimentexamples](#), the inhibitory polypeptide is an inflammatory mediator receptor, an inhibitory cytokine receptor, an immune checkpoint receptor, or a dual activator-checkpoint receptor.

[0086] In some [embodimentexamples](#), the inhibitory polypeptide is selected from the inhibitory polypeptides presented in Table 1 or Table 1.1.

[0087] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of an inhibitory polypeptide presented in Table 1 or Table 1.2.

[0088] In some [embodimentexamples](#), the transmembrane domain comprises the transmembrane domain, or a portion thereof, of a stimulatory polypeptide presented in Table 2 or Table 2.2.

[0089] In some [embodimentexamples](#), the extracellular domain and the transmembrane domain are connected by a linker. In some [embodimentexamples](#), the linker is selected from the linkers presented in Table 3.

[0090] In some [embodimentexamples](#), the immune cell is engineered to further comprise a chimeric antigen receptor (CAR). In some [embodimentexamples](#), the CAR targets a tumor antigen.

[0091] In some [embodimentexamples](#), the immune cell is engineered to further comprise a cytokine. In some [embodimentexamples](#), the cytokine can be selected from the group consisting of a chemokine, an interferon, an interleukin, a lymphokine, a tumor necrosis factor, or a variant or combination thereof. In some [embodimentexamples](#), the cytokine is an IL-15 or a fragment or variant thereof.

[0092] Also provided herein are modified cells engineered to express a protein comprising a dominant negative isoform of a protein, wherein the dominant negative isoform of the protein competes with a wild-type isoform of the protein for binding a negative signal.

[0093] In some [embodimentexamples](#), the cell is selected from the group consisting of an artificial cell, an immune cell, a fibrocyte, a mesenchymal stem cell, an induced neural stem cell, and an induced pluripotent stem cell (iPSC)-derived cell, and an erythrocyte. In some [embodimentexample](#), the immune cell is a tumor infiltrating lymphocyte (TIL). In some [embodimentexamples](#), the immune cell is selected from the group consisting of a T cell, an NK cell, an NKT cell, a type 1 innate lymphoid

cell (ILC1), an intraepithelial type 1 innate lymphoid cell (ieILC1), a type 2 innate lymphoid cell (ILC2), a type 3 innate lymphoid cell (ILC3), a lymphoid tissue inducer cell (LTi), a monocyte, a macrophage, a dendritic cell (DC), a platelet, a marrow-infiltrating lymphocyte (MIL), and a B cell. In some [embodimentexamples](#), the immune cell is an NK cell.

[0094] In some [embodimentexamples](#), the dominant negative isoform of the protein is a dominant negative isoform of an inhibitory polypeptide selected from Table 1 or Table 1.1.

[0095] In some [embodimentexamples](#), the dominant negative isoform of the protein is a dominant negative isoform of TGF-BR1. In some [embodimentexamples](#), the dominant negative isoform of TGF-BR1 is selected from the dominant negative isoforms of TGF-BR1 presented in Table 4.

[0096] In some [embodimentexamples](#), the immune cells comprising a CAR described herein are T cells (e.g., alpha beta T cells and gamma delta T cells). In some [embodimentexamples](#), the T cells are one or more of CD3⁺, CD28⁺, CD4⁺, CD8⁺, CD45RA⁺, CD25⁺ and CD45RO⁺. In some [embodimentexamples](#), the T cells are isolated tumor infiltrating lymphocytes (TIL). In some [embodimentexamples](#), the T cells are CD4⁺ T cells. In some [embodimentexamples](#), the T cells are CD8⁺ T cells. In some [embodimentexamples](#), the T cells are regulatory T cell (e.g., a CD4⁺, CD25⁺, CD62L^{hi}, GITR⁺ and FoxP3⁺ T cells). In some [embodimentexamples](#), the T cells are memory T cells (T_{CM}) (e.g., CD62L⁺, CCR7⁺, CD45RO⁻ and CD45RA⁻). In some [embodimentexamples](#), the T cells are stem cell memory T cells. In some [embodimentexamples](#), the T cells are naïve T cells. In some [embodimentexamples](#), the T cells are a mixed population of CD4⁺ T cells, CD8⁺ T cells, stem cell memory T cells and naïve T cells. In some [embodimentexamples](#), the immune cells comprising a protein described herein (e.g., a CAR) are natural killer T (NKT) cells. NKT cells recognize glycolipid antigen presented by a molecule called CD1d.

[0097] In some [embodimentexamples](#), the dominant negative isoform of the protein is a dominant negative isoform of TGF-BR2. In some [embodimentexamples](#), the dominant negative isoform of TGF-BR2 is selected from the dominant negative isoforms of TGF-BR2 presented in Table 5.

[0098] In some [embodimentexamples](#), the immune cell is engineered to further comprise a chimeric antigen receptor (CAR). In some [embodimentexamples](#), the CAR targets a tumor antigen. In some [embodimentexamples](#), the immune cell is engineered to further comprise a cytokine. In some [embodimentexamples](#), the cytokine can be selected from the group consisting of a chemokine, an interferon, an interleukin, a lymphokine, a tumor necrosis factor, or a variant or combination thereof. In some [embodimentexamples](#), the cytokine is an IL-15 or a fragment or variant thereof.

[0099] Also provided herein are modified cells engineered to express at least two proteins selected from the group consisting of: (a) a chimeric protein comprising an extracellular domain, a transmembrane domain, and an intracellular domain, wherein the extracellular domain is capable of binding a negative signal, and wherein the intracellular domain comprises an intracellular domain, or a portion thereof, of a stimulatory polypeptide; (b) a protein comprising a dominant negative isoform of a protein, wherein the dominant negative isoform of the protein competes with a wild-type isoform of the protein for binding a negative signal that prevents or decreases the activation of an immune response; and (c) a protein comprising an extracellular domain and a transmembrane domain, wherein the extracellular domain is capable of binding a negative signal, and wherein the protein lacks a fully functional intracellular domain.

[0100] In some embodiment examples, the cell is selected from the group consisting of an artificial cell, an immune cell, a fibrocyte, a mesenchymal stem cell, an induced neural stem cell, and an induced pluripotent stem cell (iPSC)-derived cell. In some embodiment examples, the immune cell is selected from the group consisting of a T cell, an NK cell, an NKT cell, a type 1 innate lymphoid cell (ILC1), an intraepithelial type 1 innate lymphoid cell (ieILC1), a type 2 innate lymphoid cell (ILC2), a type 3 innate lymphoid cell (ILC3), a lymphoid tissue inducer cell (LTi), a monocyte, a macrophage, a dendritic cell (DC), a platelet, a marrow-infiltrating lymphocyte (MIL), and a B cell. In some embodiment examples, the immune cell is a tumor infiltrating lymphocyte (TIL). In some embodiment examples, the immune cell is an NK cell.

[0101] Also provided herein are polynucleotides that include a nucleic acid sequence encoding any of the chimeric proteins, or engineered proteins (e.g., chimeric proteins) described herein. In one aspect, the present disclosure refers to a polynucleotide comprising a nucleic acid sequence encoding a chimeric protein as disclosed herein.

[0102] Also provided herein are pharmaceutical compositions that include any of the modified cells described herein, and a pharmaceutically acceptable excipient. In one aspect, the present disclosure refers to a pharmaceutical composition comprising the modified immune cell as disclosed herein, and a pharmaceutically acceptable excipient.

[0103] Also provided herein are methods of treating a subject in need of an altered immune response that include administering to the subject an effective amount of a composition comprising any of the modified cells described herein, thereby treating the subject in need of the altered immune response. In one aspect, the present disclosure refers to use of a composition comprising the modified immune cell as disclosed herein in the manufacture of a medicament for treating a subject in need of an altered immune response.

[0104] Also provided herein are methods of treating a disease or pathological condition in a subject that include administering to the subject an effective amount of a composition comprising any of the modified cell described herein, thereby treating the disease or pathological condition in the subject. In one aspect, the present disclosure refers to use of a composition comprising the modified immune cell as disclosed herein in the manufacture of a medicament for treating a disease or pathological condition in a subject.

[0105] Also provided herein are methods of treating a cancer in a subject that include administering to the subject a therapeutically effective amount of a composition comprising any of the modified cells described herein, thereby treating the cancer in the subject. In one aspect, the present disclosure refers to use of a composition comprising the modified immune cell as disclosed herein in the manufacture of a medicament for treating a cancer in a subject.

[0106] Also provided herein are methods of generating any of the modified cells described herein that include: (a) introducing a nucleic acid encoding any of the chimeric proteins or engineered proteins (e.g., chimeric proteins) described herein, into a cell; (b) culturing the cell under conditions allowing the expression of the protein in or on the cell; and (c) recovering the cell from the culture, thereby generating the modified cell. In one aspect, the present disclosure refers to a method of generating a modified immune cell, the method comprising: (a) introducing a nucleic acid encoding the chimeric protein as disclosed herein, into an immune cell; (b) culturing the immune cell under conditions allowing the expression of the protein in or on the immune cell; and (c) recovering the immune cell from the culture, thereby generating the modified immune cell.

[0107] Also provided herein are cells obtained by the methods described herein. In one aspect, the present disclosure refers to a modified immune cell obtained by the method as disclosed herein.

[0108] Also provided herein are kits that include any of the chimeric proteins or any of the engineered proteins (e.g., chimeric proteins) described herein, any of the modified cells described herein, and/or any of the nucleic acids encoding any of the chimeric proteins or the engineered proteins described herein. In one aspect, the present disclosure refers to a kit comprising a chimeric protein and/or a nucleic acid encoding the chimeric protein, wherein the chimeric protein is the chimeric protein as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0109] FIG. 1 is a general schematic of the sink, dominant negative, and signal inverter modalities of the disclosure.

[0110] FIG. 2 is a schematic of an exemplary protein of the sink modality of the disclosure.

[0111] FIG. 3 is a schematic of an exemplary protein of the dominant negative receptor modality of the disclosure.

[0112] FIG. 4 is a schematic of an exemplary chimeric protein of the signal inverter modality of the disclosure.

[0113] FIG. 5 is a graph showing that stimulation of reporter cells expressing different chimeric proteins including the extracellular domains of inhibitory receptors induces NF- κ B activation.

[0114] FIG. 6 is a graph showing that stimulation of reporter cells expressing different chimeric proteins including the extracellular domains of inhibitory receptors induces CD69 expression.

[0115] FIG. 7A is a graph showing that TGF-B1 stimulation of reporter cells expressing different chimeric proteins including the extracellular domain of TGF-BR2 induces NF- κ B activation.