SUMMARY

[0004] The present disclosure provides, among other things, methods and compositions for use in the treatment of cancer. The present disclosure is based, in part, on the surprising discovery that a targeting masked cytokines of the present disclosure specifically target a cell of interest for effective treatment of cancer without causing undesired side effects. The targeting cytokines of the present disclosure specifically bind the target cells of interest, accumulate in tumor, and be activated by tumor proteases. Furthermore, treatment with the targeting masked cytokines of the present disclosure increases the percentage of CD8 T cells in the tumor microenvironment and significantly inhibits tumor growth with minimal toxicity *in vivo.*

[0005] In one aspect, the present disclosure provides a targeted cytokine comprising: a) a targeting moiety; b) a cytokine or a fragment thereof; c) a masking moiety; and d) an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker, wherein the masking moiety binds to the cytokine or a fragment thereof, wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage, wherein the cleavable linker comprises (i) ***MPYDLYHP (SEQ ID NO: 34) or VPLSLYSG (SEQ ID NO: 42),*** (ii) ***an amino acid sequence selected from Table 1, or (iii) between 8-50 amino acid residues*** and wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides. In one example, the present disclosure provides, among other things, a targeted cytokine comprising a targeting moiety, a cytokine or fragment thereof, a masking moiety, and an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker.

[0006] In one example, the present disclosure provides, among other things, a targeted cytokine comprising a targeting moiety, a cytokine or a fragment thereof, a masking moiety, and an Fc domain comprising a first Fc polypeptide linked to the IL-2 cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker, wherein the wherein the masking moiety binds to the IL-2 cytokine or a fragment thereof, wherein the first or the second linker is a

cleavable linker such that the masking moiety releases the IL-2 cytokine or a fragment thereof upon cleavage, wherein the cleavable linker comprises a sequence selected from Table 1 and wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

[0007] In one example, the present disclosure provides, among other things, a targeted cytokine comprising a targeting moiety, a cytokine or a fragment thereof, a masking moiety, and an Fc domain comprising a first Fc polypeptide linked to the IL-2 cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker, wherein the wherein the masking moiety binds to the IL-2 cytokine or a fragment thereof, wherein the first or the second linker is a

cleavable linker such that the masking moiety releases the IL-2 cytokine or a fragment thereof upon cleavage, wherein the cleavable linker comprises MPYDLYHP (SEQ ID NO: 34) or VPLSLYSG (SEQ ID NO: 42) and wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

[0008] In some examples, the cleavable linker comprises PVSLRSGS (SEQ ID NO: 196), or GMPKDLYHAS (SEQ ID NO. 197), or RPLALWRS (SEQ ID NO: 193), or TQKPLGLS (SEQ ID NO:194), or APAGLIVPYN (SEQ ID NO:195), or PANLVAPDP (SEQ ID NO: 183), or IVGRPRHQGV (SEQ ID NO:199), or RSKYLATA (SEQ ID NO:198).

[0009] In some examples, the cleavable linker comprises MPYDLYHP (SEQ ID NO: 34), RAAAVKSP, or VPLSLYSG (SEQ ID NO: 42).

[0010] In some examples, a masking moiety binds to the cytokine or a fragment thereof. In some examples, a masking moiety comprises an agent that binds to a cytokine or a fragment thereof. In some examples, a masking moiety comprises a peptide that binds to a cytokine or a fragment thereof. In some examples, a masking moiety comprises a polypeptide that binds to a cytokine or a fragment thereof. In some examples, a masking moiety comprises a protein that binds to a cytokine or a fragment thereof. In some examples, a masking moiety comprises a nucleic acid that binds to a cytokine or a fragment thereof.

[0011] In some examples, a masking moiety comprises a Fab. In some examples, a masking moiety comprises a single chain Fv (scFv). In some examples, a masking moiety comprises a single domain antibody (VHH). In some examples, a masking moiety comprises one or more CDRs. In some examples, a masking moiety comprises a variable heavy chain (VH). In some examples, a masking moiety comprises a variable light chain (VL). In some examples, a masking moiety comprises a Fab-like bispecific antibodies (bsFab). In some examples, a masking moiety comprises a single-domain antibody-linked Fab (s-Fab). In some examples, a masking moiety comprises an antibody. In some examples, a masking moiety comprises a combination thereof. In some examples, a masking moiety comprises a Fab that binds to a cytokine. In some examples, a masking moiety comprises a single chain Fv (scFv) that binds to a cytokine. In some examples, a

masking moiety comprises a single domain antibody (VHH) that binds to a cytokine. In some examples, a masking moiety comprises one or more CDRs that bind to a cytokine. In some examples, a masking moiety comprises a variable heavy chain (VH) that binds to a cytokine. In some examples, a masking moiety comprises a variable light chain (VL) that binds to a cytokine. In some examples, a masking moiety comprises a Fab-like bispecific antibodies (bsFab) that binds to a cytokine. In some examples, a masking moiety comprises a single-domain antibody-linked Fab (s-Fab) that binds to a cytokine. In some examples, a masking moiety comprises an antibody or a fragment thereof that binds to a cytokine. In some examples, the masking moiety comprises an antibody against the cytokine or a binding fragment of the antibody.

[0012] In some examples, the masking moiety is a receptor of the cytokine. In some examples, the masking moiety is a fragment of a receptor of the cytokine. In some examples, the masking moiety is an extracellular domain (ECD) of a receptor of the cytokine.

[0013] In some examples, the masking moiety is CD121. In some examples, the masking moiety is IL-18Rα. In some examples, the masking moiety is IL-18Rβ. In some examples, the masking moiety is CD25. In some examples, the masking moiety is CD132. In some examples, the masking moiety is CD124. In some examples, the masking moiety is CD213a13. In some examples, the masking moiety is CD132. In some examples, the masking moiety is CD127. In some examples, the masking moiety is IL-9R. In some examples, the masking moiety is CD213a1. In some examples, the masking moiety is CD213a2. In some examples, the masking moiety is CD1243. In some examples, the masking moiety is CD132. In some examples, the masking moiety is IL-15Ra. In some examples, the masking moiety is CDw131. In some examples, the masking moiety is CDw125. In some examples, the masking moiety is CD131. In some examples, the masking moiety is CD116. In some examples, the masking moiety is CD126. In some examples, the masking moiety is CD130. In some examples, the masking moiety is IL-11Ra. In some examples, the masking moiety is CD114. In some examples, the masking moiety CD212. In some examples, the masking moiety is LIFR. In some

examples, the masking moiety is OSMR. In some examples, the masking moiety is IL-20Rα. In some examples, the masking moiety is IL-20Rβ. In some examples, the masking moiety is IL-14R. In some examples, the masking moiety is CD4. In some examples, the masking moiety is CDw127. In some examples, the masking moiety is CD118. In some examples, the masking moiety is CDw119. In some examples, the masking moiety is CD40. In some examples, the masking moiety is LTβR. In some examples, the masking moiety is CD120a. In some examples, the masking moiety is CD120b. In some examples, the masking moiety is CDw137. In some examples, the masking moiety is BCMA. In some examples, the masking moiety is TACI. In some examples, the masking moiety is CD27. In some examples, the masking moiety is CD30. In some examples, the masking moiety is CD95. In some examples, the masking moiety is GITR. In some examples, the masking moiety is LTbR. In some examples, the masking moiety is HVEM. In some examples, the masking moiety is OX40. In some examples, the masking moiety is TRAILR1-4. In some examples, the masking moiety is Apo3. In some examples, the masking moiety is RANK. In some examples, the masking moiety is OPG. In some examples, the masking moiety is TGF-βR1. In some examples, the masking moiety is TGF-βR2. In some examples, the masking moiety is TGF-βR3. In some examples, the masking moiety is CD115. In some examples, the masking moiety is CDw136.

[0014] In some examples, the masking moiety is CD122. In some examples, the CD122 is an engineered CD122 polypeptide or a fragment thereof comprising one or more mutations relative to a wild-type CD122 amino acid sequence.

[0015] In some examples, the engineered CD122 comprises F8C mutation. In some examples, the engineered CD122 comprises A94C mutation. In some examples, the engineered CD122 comprises L106C mutation. In some examples, the engineered CD122 comprises C122S mutation. In some examples, the engineered CD122 comprises C122V mutation. In some examples, the engineered CD122 comprises C122A mutation. In some examples, the engineered CD122 comprises N123C mutation. In some examples, the engineered CD122 comprises N123Q mutation. In some examples, the engineered CD122 comprises C168V mutation. In

some examples, the engineered CD122 comprises C168A mutation. In some examples, the engineered CD122 comprises C168S mutation. In some examples, the engineered CD122 comprises L169C mutation. In some examples, the engineered CD122 comprises Q177C mutation. In some examples, the engineered CD122 comprises V184C mutation. In some examples, the engineered CD122 comprises S195C mutation. In some examples, the engineered CD122 comprises R204C mutation.

[0016] In some examples, the engineered CD122 comprises C122S and C168S mutations. In some examples, the engineered CD122 comprises C122A and N123C mutations.

[0017] In some examples, the engineered CD122 comprises C122V and C168Vmutations. In some examples, the engineered CD122 comprises C122A and C168V mutations. In some examples, the engineered CD122 comprises C122V and C168A mutations. In some examples, the engineered CD122 comprises C122V and N123C mutations. In some examples, the engineered CD122 comprises C122A and C168A mutations. In some examples, the engineered CD122 comprises V117C, N123Q and C168A mutations. In some examples, the engineered CD122 comprises N123Q, C168A, and L169C mutations. In some examples, the engineered CD122 comprises L106C, C122A, C168A and S195C mutations. In some examples, the engineered CD122 comprises L106C, C122A, C168A and V184C mutations. In some examples, the engineered CD122 comprises C122A, C168A, V184C, and S195C mutations. In some examples, the engineered CD122 comprises C122A, C168A, Q177C, and R204C mutations. In some examples, the engineered CD122 comprises L106C, C122V, C168V, and S195C mutations. In some examples, the engineered CD122 comprises F8C, A94C, C122V, and C168V mutations.

[0018] In some examples, the engineered CD122 polypeptide or a fragment thereof comprises a mutation at amino acid 122 and 168 relative to SEQ ID NO: 15, wherein the engineered CD122 has improved stability compared to a wild-type CD122. In some examples, the engineered CD122 polypeptide or a fragment thereof comprises a mutation to facilitate disulfide bond formation. In some examples, the engineered CD122 polypeptide or a fragment thereof comprises a mutation that creates a salt bridge.

[0019] In some examples, the masking moiety comprises the extracellular domain of human IL-12Rβ1 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12. In some examples, the masking moiety comprises residues 24 to 237 of human IL-12Rβ1. In some examples, the masking moiety comprises a sequence having SEQ ID NO: 126. In some examples, the masking moiety comprises residues 24 to 545 of human IL-12Rβ1. In some examples, the masking moiety comprises a sequence having SEQ ID NO: 127.

[0020] In some examples, the masking moiety comprises the extracellular domain of human IL-12Rβ2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12. In some examples, the masking moiety comprises residues 24 to 212 of human IL-12Rβ2. In some examples, the masking moiety comprises a sequence having SEQ ID NO: 128. In some examples, the masking moiety comprises residues 24 to 222 of human IL-12Rβ2. In some examples, the masking moiety comprises a sequence having SEQ ID NO: 129. In some examples, the masking moiety comprises residues 24 to 227 of human IL-12Rβ2. In some examples, the masking moiety comprises a sequence having SEQ ID NO: 133. In some examples, the masking moiety comprises residues 24 to 319 of human IL-12Rβ2. In some examples, the masking moiety comprises a sequence having SEQ ID NO: 130.

[0021] In some examples, the masking moiety comprises at least one amino acid modification as compared to the sequence of SEQ ID NO: 130. In some examples, the masking moiety comprises at least one amino acid modification as compared to the sequence of SEQ ID NO: 130, optionally wherein said modifications are cysteine substitution mutations. In some examples, the masking moiety comprises SEQ ID NO: 131. In some examples, the masking moiety comprises residues 24 to 622 of human IL-12Rβ2. In some examples, the masking moiety comprises a sequence having SEQ ID NO: 132.

[0022] In some examples, a cytokine is IL-1 or a variant thereof. In some examples, a cytokine is IL-2 or a variant thereof. In some examples, a cytokine is IL-3 or a variant thereof. In some examples, a cytokine is IL-4 or a variant thereof. In some examples, a cytokine is IL-5 or a variant thereof. In some examples, a

cytokine is IL-6 or a variant thereof. In some examples, a cytokine is IL-7 or a variant thereof. In some examples, a cytokine is IL-9 or a variant thereof. In some examples, a cytokine is IL-10 or a variant thereof. In some examples, a cytokine is IL-11 or a variant thereof. In some examples, a cytokine is IL-12 or a variant thereof. In some examples, a cytokine is IL-13 or a variant thereof. In some examples, a cytokine is IL-14 or a variant thereof. In some examples, a cytokine is IL-15 or a variant thereof. In some examples, a cytokine is IL-16 or a variant thereof. In some examples, a cytokine is IL-17 or a variant thereof. In some examples, a cytokine is IL-20 or a variant thereof. In some examples, a cytokine is TNF-α or a variant thereof. In some examples, a cytokine TNF-β or a variant thereof. In some examples, a cytokine is CXCL8 (IL-18) or a variant thereof. In some examples, a cytokine is G-CSF or a variant thereof. In some examples, a cytokine is CXCL8 (IL-18) or a variant thereof. In some examples, a cytokine is GM-CSF or a variant thereof. In some examples, a cytokine is LIF or a variant thereof. In some examples, a cytokine is OSM or a variant thereof. In some examples, a cytokine is IFN-α or a variant thereof. In some examples, a cytokine is IFN-β or a variant thereof. In some examples, a cytokine is IFN-γ or a variant thereof. In some examples, a cytokine is CD154 or a variant thereof. In some examples, a cytokine is LT-β or a variant thereof. In some examples, a cytokine is 4-1BBL or a variant thereof. In some examples, a cytokine is APRIL or a variant thereof. In some examples, a cytokine is CD153 or a variant thereof. In some examples, a cytokine is CD70 or a variant thereof. In some examples, a cytokine is CD178 or a variant thereof. In some examples, a cytokine is GITRL or a variant thereof. In some examples, a cytokine is LIGHT or a variant thereof. In some examples, a cytokine is OX40L or a variant thereof. In some examples, a cytokine is TALL-1 or a variant thereof. In some examples, a cytokine is TRAIL or a variant thereof. In some examples, a cytokine is TWEAK or a variant thereof. In some examples, a cytokine is TRANCE or a variant thereof. In some examples, a cytokine is TGF-β or a variant thereof. In some examples, a cytokine is M-CSF or a variant thereof. In some examples, a cytokine is MSP or a variant thereof.

[0023] In some examples, the IL-2 is a modified IL-2 cytokine or functional fragment thereof compared to the sequence of a mature IL-2 having SEQ ID NO: 13. In

some examples, the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A relative to the sequence of a mature IL-2 having SEQ ID NO: 13. In some examples, the modified IL-2 cytokine or functional fragment thereof comprises modifications F42A relative to the sequence of a mature IL-2 having SEQ ID NO: 13. In some examples, the modified IL-2 cytokine or functional fragment thereof comprises modifications Y45A relative to the sequence of a mature IL-2 having SEQ ID NO: 13. In some examples, the modified IL-2 cytokine or functional fragment thereof comprises modifications E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 13. In some examples, the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 13. In some examples, the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 13.

[0024] In some examples, the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 13. In some examples, the modified IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 14.

[0025] In some examples, the modified IL-2 cytokine or functional fragment thereof comprises modifications that reduce or eliminate binding to CD25.

[0026] In some examples, the modified IL-2 cytokine or functional fragment thereof comprises modifications that enhance binding to CD25.

[0027] In some examples, the modified IL-2 cytokine or functional fragment thereof comprises modifications that reduce or eliminate binding to CD122.

[0028] In some examples, the modified IL-2 cytokine or functional fragment thereof comprises modifications that enhance binding to CD122.

[0029] In some examples, the IL-15 polypeptide comprises an amino acid sequence of SEQ ID NO: 93 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 93. In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid

sequence having one or more amino acid substitutions at positions D22 as compared to the amino acid sequence of SEQ ID NO: 93. In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions E46 as compared to the amino acid sequence of SEQ ID NO: 93. In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions E53 as compared to the amino acid sequence of SEQ ID NO: 93. In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions N71 as compared to the amino acid sequence of SEQ ID NO: 93. In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions N79 as compared to the amino acid sequence of SEQ ID NO: 93. In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions N112 as compared to the amino acid sequence of SEQ ID NO: 93.

[0030] In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions D22, E46, or E53 as compared to the amino acid sequence of SEQ ID NO: 93. In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71 and N79 as compared to the amino acid sequence of SEQ ID NO: 93. In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71 and N112 as compared to the amino acid sequence of SEQ ID NO: 93. In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N79 and N112 as compared to the amino acid sequence of SEQ ID NO: 93. In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions D22, E46, E53, N71, N79, or N112 as compared to the amino acid sequence of SEQ ID NO: 93. In some examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 94.

[0031] In some examples, the IL-12 polypeptide or functional fragment thereof comprises an IL-12p40 polypeptide or functional fragment thereof covalently linked to an IL-12p35 polypeptide or functional fragment thereof. In some examples, the IL-12p40 – IL-12p35 linker is between 2 and 40 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is between 4 and 30 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is between 5 and 20 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is between 7 and 15 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is between 10 and 12 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 2 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 2 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 2 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 2 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 2 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 2 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 3 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 4 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 5 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 6 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 7 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 8 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 9 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 10 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 11 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 12 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 13 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 14 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 15 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 16 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 17 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 18 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 19 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 20 amino acids in length. In some

examples, the IL-12p40 – IL-12p35 linker is 21 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 22 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 23 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 24 amino acids in length. In some examples, the IL-12p40 – IL-12p35 linker is 25 amino acids in length.

[0032] In some examples, the IL-12p40 – IL-12p35 linker comprises amino acid residues A, P, G and S. In some examples, the IL-12p40 – IL-12p35 linker is rich in amino acid residues G and S. In some examples, the IL-12p40 – IL-12p35 linker comprises SEQ ID NO: 119 (GGGGSGGGGSGGGGS). In some examples the IL-12p40 polypeptide comprises SEQ ID NO: 111. In some examples the IL-12p40 polypeptide comprises an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 111. In some examples the IL-12p40 polypeptide comprises an amino acid sequence having one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 111. In some examples the IL-12p40 polypeptide comprises an amino acid sequence having two amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 111. In some examples the IL-12p40 polypeptide comprises an amino acid sequence having three amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 111. In some examples the IL-12p40 polypeptide comprises an amino acid sequence having four amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 111. In some examples the IL-12p40 polypeptide comprises an amino acid sequence having five amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 111.

[0033] In some examples, IL-12p40 polypeptide comprises at least one amino acid modification to the GAG-binding domain (KSKREKKDRV) as compared to the amino acid sequence of SEQ ID NO: 111.

[0034] In some examples, the IL-12p40 polypeptide comprises SEQ ID NO: 115. In some examples, the IL-12p40 polypeptide comprises SEQ ID NO: 116.

[0035] In some examples, IL-12p40 polypeptide comprises an amino acid sequence having one or more cysteine substitution mutations as compared to the amino acid sequence of SEQ ID NO: 111. In some examples, the IL-12p40 polypeptide

comprises SEQ ID NO: 117. In some examples, the IL-12p40 polypeptide comprises SEQ ID NO: 118.

[0036] In some examples, the IL-12p35 polypeptide comprises SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having at least two amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having at least three amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having at least four amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having at least five amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having at least six amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having at least seven amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having at least eight amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having at least nine amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having at least ten amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112.

[0037] In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having two amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide

comprises an amino acid sequence having three amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having four amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having five amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having six amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having seven amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having eight amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having nine amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112. In some examples, the IL-12p35 polypeptide comprises an amino acid sequence having ten amino acid modifications as compared to the amino acid sequence of SEQ ID NO: 112.

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQ
VKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAK
NYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSV
ECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSR
QVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASIS
VRAQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPCLHHS
QNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNS
RETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLD
QNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMS
YLNAS (**SEQ ID NO: 150**)

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQ
VKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAK
NYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSV
ECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK

PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKDNTERVFTDKTSATVICRKNA
SISVRAQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPCLH
HSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCL
NSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIF
LDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRV
MSYLNAS (**SEQ ID NO: 151**)

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQ
VKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAK
NYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSV
ECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSR
QVEVSWEYPDTWSTPHSYFSLTFCVQVQGKDNTEGRVFTDKTSATVICRKNASISVR
AQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPCLHHSQNL
LRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRET
SFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNM
LAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNA
S (**SEQ ID NO: 152**)

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQ
VKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAK
NYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSV
ECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSR
QVEVSWEYPDTWSTPHSYFSLTFSVQVQGKSKREKKDRVFTDKTSATVICRKNASIS
VRAQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPCLHHS
QNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNS
RETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLD
QNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMS
YLNAS (**SEQ ID NO: 153**)

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQ
VKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAK
NYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSV
ECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSR
QVEVSWEYPDTWSTPHSYFSLTFSVQVQGKDNTEGRVFTDKTSATVICR

KNASISVRAQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFP CLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNE SCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKR QIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTID RVMSYLNAS (**SEQ ID NO: 154**)

[0038] In some examples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 150. In some examples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 151. In some examples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 152. In some examples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 153. In some examples, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 154.

[0039] In some examples the first linker or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage.

[0040] In some examples, the cleavable linker comprises MPYDLYHP. In some examples, the cleavable linker comprises VPLSLYSG (SEQ ID NO: 42). In some examples, the cleavable linker comprises MPYDLYHP (SEQ ID NO: 34) and VPLSLYSG (SEQ ID NO: 42). In some examples, the cleavable linker comprises DSGGFMLT (SEQ ID NO: 35). In some examples, the cleavable linker comprises HEQLTV (SEQ ID NO: 36). In some examples, the cleavable linker comprises RAAAVKSP (SEQ ID NO: 37). In some examples, the cleavable linker comprises VPLSLY (SEQ ID NO: 38). In some examples, the cleavable linker comprises DLLAVVAAS (SEQ ID NO: 39). In some examples, the cleavable linker comprises ISSGLLSGRS (SEQ ID NO: 40).

[0041] In some examples, the cleavable linker comprises between 6-60 amino acid residues. In some examples, the cleavable linker comprises between 8-50 amino acid residues. In some examples, the cleavable linker comprises between 8-30 amino acid residues. In some examples, the cleavable linker comprises between 8-25 amino acid residues. In some examples, the cleavable linker comprises 8 amino acid residues. In some examples, the cleavable linker comprises 9 amino acid residues. In some

examples, the cleavable linker comprises 10 amino acid residues. In some examples, the cleavable linker comprises 11 amino acid residues. In some examples, the cleavable linker comprises 12 amino acid residues. In some examples, the cleavable linker comprises 13 amino acid residues. In some examples, the cleavable linker comprises 14 amino acid residues. In some examples, the cleavable linker comprises 15 amino acid residues. In some examples, the cleavable linker comprises 16 amino acid residues. In some examples, the cleavable linker comprises 17 amino acid residues. In some examples, the cleavable linker comprises 18 amino acid residues. In some examples, the cleavable linker comprises 19 amino acid residues. In some examples, the cleavable linker comprises 20 amino acid residues. In some examples, the cleavable linker comprises 21 amino acid residues. In some examples, the cleavable linker comprises 22 amino acid residues. In some examples, the cleavable linker comprises 23 amino acid residues. In some examples, the cleavable linker comprises 24 amino acid residues. In some examples, the cleavable linker comprises 25 amino acid residues.

[0042] In some examples, the cleavable linker comprises a proteolytically cleavable peptide. In some examples, the cleavable linker comprises a proteolytically cleavable peptide flanked on the N-terminus by a spacer domain. In some examples, the cleavable linker comprises a proteolytically cleavable peptide flanked on the C-terminus by a spacer domain. In some examples, the cleavable linker comprises a proteolytically cleavable peptide flanked on both sides by a spacer domain. In some examples, the spacer domain is between 2 and 20 amino acids in length. In some examples, the spacer domain is between 3 and 10 amino acids in length. In some examples, the spacer domain is between 3 and 6 amino acids in length. In some examples, the spacer domain is 2 amino acids in length. In some examples, the spacer domain is 3 amino acids in length. In some examples, the spacer domain is 4 amino acids in length. In some examples, the spacer domain is 5 amino acids in length. In some examples, the spacer domain is 6 amino acids in length. In some examples, the spacer domain is 7 amino acids in length. In some examples, the spacer domain is 8 amino acids in length. In some examples, the spacer domain is 9 amino acids in length. In some examples, the spacer domain is 10 amino acids in

length. In some examples, the spacer domain is 11 amino acids in length. In some examples, the spacer domain is 12 amino acids in length. In some examples, the spacer domain is 13 amino acids in length. In some examples, the spacer domain is 14 amino acids in length. In some examples, the spacer domain is 15 amino acids in length.

[0043] In some examples, the targeting moiety comprises at least one antigen binding domain. In some examples, the targeting moiety comprises a first antigen binding domain and a second antigen binding domain. In some examples, the targeting moiety comprises a first antigen binding domain, a second antigen binding domain, and a third antigen binding domain. In some examples, the targeting moiety comprises a first antigen binding domain, a second antigen binding domain, a third antigen binding domain, and a fourth antigen binding domain. In some examples the first and second antigen binding domains specifically bind to the same target. In some examples the first, second, and a third antigen binding domains specifically bind to the same target. In some examples the first, second, third, and fourth antigen binding domains specifically bind to the same target. In some examples the first and second antigen binding domains specifically bind to different targets. In some examples the first, second, and third antigen binding domains each specifically bind to different targets. In some examples the first, second, third, and fourth antigen binding domains each specifically bind to different targets.

[0044] In some examples, the first and second antigen binding domains comprise different amino acid sequences. In some examples, the first and second antigen binding domains comprise same amino acid sequences. In some examples, the first, second, and third antigen binding domains comprise different amino acid sequences. In some examples, the first, second, and third antigen binding domains comprise same amino acid sequences. In some examples, the first, second, third, and fourth, antigen binding domains comprise different amino acid sequences. In some examples, the first, second, third, and fourth antigen binding domains comprise same amino acid sequences.

[0045] In some examples, the first Fc polypeptide comprises a first IgG1 Fc domain or a fragment thereof. In some examples, the first Fc polypeptide comprises a

first IgG2 Fc domain or a fragment thereof. In some examples, the first Fc polypeptide comprises a first IgG3 Fc domain or a fragment thereof. In some examples, the first Fc polypeptide comprises a first IgG4 Fc domain or a fragment thereof. In some examples, the second Fc polypeptide comprises a second IgG1 Fc domain or a fragment thereof. In some examples, the second Fc polypeptide comprises a second IgG2 Fc domain or a fragment thereof. In some examples, the second Fc polypeptide comprises a second IgG3 Fc domain or a fragment thereof. In some examples, the second Fc polypeptide comprises a second IgG4 Fc domain or a fragment thereof.

[0046] In some examples, the first Fc domain contain one or more modifications that promote the non-covalent association of the first and the second Fc polypeptide. In some examples, the second Fc domain contain one or more modifications that promote the non-covalent association of the first and the second Fc polypeptide. In some examples, the first and or the second Fc domains each contain one or more modifications that promote the non-covalent association of the first and the second Fc polypeptide. In some examples, the first and the second Fc domains each contain one or more modifications that promote the non-covalent association of the first and the second Fc polypeptide.

[0047] In some examples, the first Fc domain comprises Y349C mutation. In some examples, the first Fc domain comprises T366S mutation. In some examples, the first Fc domain comprises L368A mutation. In some examples, the first Fc domain comprises Y407V mutation. In some examples, the first Fc domain comprises N297A mutation. In some examples, the first Fc domain comprises S354C mutation. In some examples, the first Fc domain comprises T366W mutation. In some examples, the first Fc domain comprises I253A mutation. In some examples, the second Fc domain comprises Y349C mutation. In some examples, the second Fc domain comprises T366S mutation. In some examples, the second Fc domain comprises L368A mutation. In some examples, the second Fc domain comprises Y407V mutation. In some examples, the second Fc domain comprises N297A mutation. In some examples, the second Fc domain comprises S354C mutation. In

some examples, the second Fc domain comprises T366W mutation. In some examples, the second Fc domain comprises I253A mutation.

[0048] In some examples, the first Fc domain comprises Y349C and T366S mutations. In some examples, the first Fc domain comprises Y349C, T366S, and L368A mutations. In some examples, the first Fc domain comprises Y349C and L368A mutations. In some examples, the first Fc domain comprises Y349C, T366S, L368A, and Y407V mutations. In some examples, the first Fc domain comprises Y349C, T366S, and Y407V mutations. In some examples, the first Fc domain comprises T366S, L368A, and Y407V mutations. In some examples, the first Fc domain comprises Y349C, L368A, and Y407V mutations. In some examples, the first Fc domain comprises Y349C, T366S, L368A, Y407V, and N297A mutations. In some examples, the first Fc domain comprises S354C and T366W mutations. In some examples, the first Fc domain comprises S354C and N297A mutations. In some examples, the first Fc domain comprises T366W and N297A mutations. In some examples, the first Fc domain comprises S354C, T366W and N297A mutations. In some examples, the second Fc domain comprises Y349C and T366S mutations. In some examples, the second Fc domain comprises Y349C, T366S, and L368A mutations. In some examples, the second Fc domain comprises Y349C and L368A mutations. In some examples, the second Fc domain comprises Y349C, T366S, L368A, and Y407V mutations. In some examples, the second Fc domain comprises Y349C, T366S, and Y407V mutations. In some examples, the second Fc domain comprises T366S, L368A, and Y407V mutations. In some examples, the second Fc domain comprises Y349C, L368A, and Y407V mutations. In some examples, the second Fc domain comprises Y349C, T366S, L368A, Y407V, and N297A mutations. In some examples, the second Fc domain comprises S354C and T366W mutations. In some examples, the second Fc domain comprises S354C and N297A mutations. In some examples, the second Fc domain comprises T366W and N297A mutations. In some examples, the second Fc domain comprises S354C, T366W and N297A mutations.

[0049] In some examples, the first Fc domain comprises Y349C, T366S, L368A, Y407V, and N297A mutations and the second Fc domain comprises S354C,

T366W and N297A mutations. In some examples, the second Fc domain comprises Y349C, T366S, L368A, Y407V, and N297A mutations and the first Fc domain comprises S354C, T366W and N297A mutations.

[0050] In some examples, the first Fc domain comprises Y349C, T366S, L368A, Y407V, and I253A mutations and the second Fc domain comprises S354C, T366W and I253A mutations. In some examples, the second Fc domain comprises Y349C, T366S, L368A, Y407V, and I253A mutations and the first Fc domain comprises S354C, T366W and I253A mutations.

[0051] In some examples, the first Fc domain comprises Y349C, T366S, L368A, Y407V, N297A, and I253A mutations and the second Fc domain comprises S354C, T366W, N297A and I253A mutations. In some examples, the second Fc domain comprises Y349C, T366S, L368A, Y407V, N297A, I253A mutations and the first Fc domain comprises S354C, T366W, N297A and I253A mutations.

[0052] In some examples, the first Fc polypeptide comprises a first CH3 domain comprising a modification that reduces or eliminates binding to Protein A. In some examples, the second Fc polypeptide comprises a second CH3 domain comprising a modification that reduces or eliminates binding to Protein A. In some examples, the first Fc polypeptide comprises a first CH3 domain that binds to Protein A. In some examples, the second Fc polypeptide comprises a second CH3 domain that binds to Protein A.

[0053] In some examples, the first Fc polypeptide comprises a first CH3 domain comprising a modification that reduces or eliminates binding to Protein A and the second Fc polypeptide comprises a second CH3 domain that binds to Protein A. In some examples, the second Fc polypeptide comprises a second CH3 domain comprising a modification that reduces or eliminates binding to Protein A and the first Fc polypeptide comprises a first CH3 domain that binds to Protein A.

[0054] In some examples, the CH3 domain that binds to Protein A is a human IgG1 sequence. In some examples, the CH3 domain that binds to Protein A is a human IgG2 sequence. In some examples, the CH3 domain that binds to Protein A is a human IgG4 sequence.

[0055] In some examples, the first CH3 domain is a human IgG1 sequence comprising a modification at position H435 according to Kabat numbering. In some examples, the first CH3 domain is a human IgG1 sequence comprising a modification at position Y436 according to Kabat numbering. In some examples, the first CH3 domain is a human IgG1 sequence comprising modifications at positions H435 and Y436 according to Kabat numbering. In some examples, the first CH3 domain is a human IgG1 sequence comprising a H435R modification according to Kabat numbering. In some examples, the first CH3 domain is a human IgG1 sequence comprising an Y436F modification. In some examples, the first CH3 domain is a human IgG1 sequence comprising modifications H435R and Y436F according to Kabat numbering.

[0056] In some examples, the first CH3 domain is a human IgG2 sequence comprising a modification at position H435 according to Kabat numbering. In some examples, the first CH3 domain is a human IgG2 sequence comprising a modification at position Y436 according to Kabat numbering. In some examples, the first CH3 domain is a human IgG2 sequence comprising modifications at positions H435 and Y436 according to Kabat numbering. In some examples, the first CH3 domain is a human IgG2 sequence comprising a H435R modification according to Kabat numbering. In some examples, the first CH3 domain is a human IgG2 sequence comprising an Y436F modification. In some examples, the first CH3 domain is a human IgG2 sequence comprising modifications H435R and Y436F according to Kabat numbering.

[0057] In some examples, the first CH3 domain is a human IgG3 sequence comprising a modification at position H435 according to Kabat numbering. In some examples, the first CH3 domain is a human IgG3 sequence comprising a modification at position Y436 according to Kabat numbering. In some examples, the first CH3 domain is a human IgG3 sequence comprising modifications at positions H435 and Y436 according to Kabat numbering. In some examples, the first CH3 domain is a human IgG3 sequence comprising a H435R modification according to Kabat numbering. In some examples, the first CH3 domain is a human IgG3 sequence comprising an Y436F modification. In some examples, the first CH3 domain is a human IgG3 sequence comprising modifications H435R and Y436F according to Kabat numbering.

[0058] In some examples, the second CH3 domain is a human IgG1 sequence comprising a modification at position H435 according to Kabat numbering. In some examples, the second CH3 domain is a human IgG1 sequence comprising a modification at position Y436 according to Kabat numbering. In some examples, the second CH3 domain is a human IgG1 sequence comprising modifications at positions H435 and Y436 according to Kabat numbering. In some examples, the second CH3 domain is a human IgG1 sequence comprising a H435R modification according to Kabat numbering. In some examples, the second CH3 domain is a human IgG1 sequence comprising an Y436F modification. In some examples, the second CH3 domain is a human IgG1 sequence comprising modifications H435R and Y436F according to Kabat numbering.

[0059] In some examples, the second CH3 domain is a human IgG2 sequence comprising a modification at position H435 according to Kabat numbering. In some examples, the second CH3 domain is a human IgG2 sequence comprising a modification at position Y436 according to Kabat numbering. In some examples, the second CH3 domain is a human IgG2 sequence comprising modifications at positions H435 and Y436 according to Kabat numbering. In some examples, the second CH3 domain is a human IgG2 sequence comprising a H435R modification according to Kabat numbering. In some examples, the second CH3 domain is a human IgG2 sequence comprising an Y436F modification. In some examples, the second CH3 domain is a human IgG2 sequence comprising modifications H435R and Y436F according to Kabat numbering.

[0060] In some examples, the second CH3 domain is a human IgG3 sequence comprising a modification at position H435 according to Kabat numbering. In some examples, the second CH3 domain is a human IgG3 sequence comprising a modification at position Y436 according to Kabat numbering. In some examples, the second CH3 domain is a human IgG3 sequence comprising modifications at positions H435 and Y436 according to Kabat numbering. In some examples, the second CH3 domain is a human IgG3 sequence comprising a H435R modification according to Kabat numbering. In some examples, the second CH3 domain is a human IgG3 sequence comprising an Y436F modification. In some examples, the second CH3 domain is a

human IgG3 sequence comprising modifications H435R and Y436F according to Kabat numbering.

[0061] In some examples, the first Fc domain comprises SEQ ID NO: 85. In some examples, the first Fc domain comprises SEQ ID NO: 80. In some examples, the second Fc domain comprises SEQ ID NO: 85. In some examples, the second Fc domain comprises SEQ ID NO: 80. In some examples, the first Fc domain comprises SEQ ID NO: 85 and the second Fc domain comprises SEQ ID NO: 80. In some examples, the second Fc domain comprises SEQ ID NO: 85 and the first Fc domain comprises SEQ ID NO: 80.

[0062] In some examples, the first CH3 domain comprises a human IgG3 sequence. In some examples, the second CH3 domain comprises a human IgG3 sequence.

[0063] In some examples, the first Fc domain comprises SEQ ID NO: 84. In some examples, the first Fc domain comprises SEQ ID NO: 80. In some examples, the second Fc domain comprises SEQ ID NO: 84. In some examples, the second Fc domain comprises SEQ ID NO: 80. In some examples, the first Fc domain comprises SEQ ID NO: 84 and the second Fc domain comprises SEQ ID NO: 80. In some examples, the second Fc domain comprises SEQ ID NO: 84 and the first Fc domain comprises SEQ ID NO: 80.

[0064] In some examples, the first antigen binding domain comprises VH, and the second antigen binding domain comprises a VL. In some examples, the first antigen binding domain comprises three HCDRs and the second antigen binding domain comprises three LCDRs.

[0065] In some examples, the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptide. In some examples, the targeting moiety is linked to the Fc domain through the first Fc polypeptide. In some examples, the targeting moiety is linked to the Fc domain through the second Fc polypeptide. In some examples, the targeting moiety is linked to the Fc domain through both the first and the second Fc polypeptide. In some examples, the targeting moiety is linked to N-terminus of the Fc domain through one or both of the first and

second Fc polypeptide. In some examples, the targeting moiety is linked to N-terminus of the Fc domain through the first Fc polypeptide. In some examples, the targeting moiety is linked to N-terminus of the Fc domain through the second Fc polypeptide. In some examples, the targeting moiety is linked to N-terminus of the Fc domain through both of the first and the second Fc polypeptide. In some examples, the targeting moiety is linked to C-terminus of the Fc domain through one or both of the first and second Fc polypeptide. In some examples, the targeting moiety is linked to C-terminus of the Fc domain through the first Fc polypeptide. In some examples, the targeting moiety is linked to C-terminus of the Fc domain through the second Fc polypeptide. In some examples, the targeting moiety is linked to C-terminus of the Fc domain through both of the first and the second Fc polypeptide.

[0066] In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to the Fc domain through one or both of the first and second Fc polypeptide. In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to the Fc domain through the first Fc polypeptide. In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to the Fc domain through the second Fc polypeptide. In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to the Fc domain through both of the first and the second Fc polypeptide. In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to N-terminus of the Fc domain through one or both of the first and second Fc polypeptide. In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to N-terminus of the Fc domain through the first Fc polypeptide. In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to N-terminus of the Fc domain through the second Fc polypeptide. In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to N-terminus of the Fc domain through both of the first and the second Fc polypeptide. In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to C-terminus of the Fc domain through one or both of the first and second Fc polypeptide. In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to C-terminus of the Fc domain through the first Fc polypeptide. In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to C-terminus

of the Fc domain through the second Fc polypeptide. In some examples, the targeting moiety comprises a variable heavy region (VH) and is linked to C-terminus of the Fc domain through both of the first and the second Fc polypeptide.

[0067] In some examples, the targeting moiety comprises a Fab. In some examples, the targeting moiety comprises a Fab having HCDR1 of SEQ ID NO: 4 (GYTFTNYY), HCDR2 of SEQ ID NO: 5 (INPSNGGT), HCDR3 SEQ ID NO: 6 (ARRDYRFDMGFDY), LCDR1 of SEQ ID NO: 9 (KGVSTSGYSY), LCDR2 of SEQ ID NO: 10 (LAS), LCDR3 of SEQ ID NO: 11 (QHSRDLPLT).

[0068] In some example, the targeting moiety comprises a variable heavy chain (VH) of SEQ ID NO: 1 and a variable light chain (VL) of SEQ ID NO: 7. In some examples, the targeting moiety comprises a heavy chain of SEQ ID NO: 2 and a light chain of SEQ ID NO: 8.

[0069] In some examples, a cytokine is linked to a first Fc polypeptide through a non-cleavable linker. In some examples, the N-terminus of a cytokine is linked to the C-terminus of a first Fc polypeptide through a non-cleavable linker. In some examples, the C-terminus of a cytokine is linked to the N-terminus of a first Fc polypeptide through a non-cleavable linker. In some examples, a cytokine is linked to a first Fc polypeptide through a cleavable linker. In some examples, the N-terminus of a cytokine is linked to the C-terminus of a first Fc polypeptide through a cleavable linker. In some examples, the C-terminus of a cytokine is linked to the N-terminus of a first Fc polypeptide through a cleavable linker.

[0070] In some examples, a masking moiety is linked to a second Fc polypeptide through a non-cleavable linker. In some examples, the N-terminus a masking moiety is linked to the C-terminus of a second Fc polypeptide through a non-cleavable linker. In some examples, the C-terminus a masking moiety is linked to the N-terminus of a second Fc polypeptide through a non-cleavable linker. In some examples, a masking moiety is linked to a second Fc polypeptide through a cleavable linker. In some examples, the N-terminus a masking moiety is linked to the C-terminus of a second Fc polypeptide through a cleavable linker. In some examples, the C-terminus a masking moiety is linked to the N-terminus of a second Fc polypeptide through a cleavable linker.

[0071] In some examples, the C-terminus of the first antigen binding domain is linked to the N-terminus of the first Fc polypeptide. In some examples, the C-terminus of the first Fc polypeptide is linked to the N-terminus of the cytokine or a fragment thereof. In some examples, the C-terminus of the second antigen binding domain is linked to the N-terminus of the second Fc polypeptide. In some examples, the C-terminus of the second Fc polypeptide is linked to the N-terminus of the masking moiety.

[0072] In some examples, a targeting moiety comprises an agent that specifically binds to a target. In some examples, a targeting moiety comprises a peptide that specifically binds to a target. In some examples, a targeting moiety comprises a polypeptide that specifically binds to a target. In some examples, a targeting moiety comprises a polypeptide that specifically binds to a target. In some examples, a targeting moiety comprises a protein that specifically binds to a target. In some examples, a targeting moiety comprises a nucleic acid that specifically binds to a target.

[0073] In some examples, a targeting moiety comprises a Fab, a single chain Fv (scFv), a single domain antibody (VHH), one or more CDRs, a variable heavy chain (VH), a variable light chain (VL), a Fab-like bispecific antibodies (bsFab), a single-domain antibody-linked Fab (s-Fab), an antibody, or a combination thereof. In some examples, a targeting moiety comprises a Fab. In some examples, a targeting moiety comprises a single chain Fv (scFv). In some examples, a targeting moiety comprises a single domain antibody (VHH). In some examples, a targeting moiety comprises one or more CDRs. In some examples, a targeting moiety comprises a variable heavy chain (VH). In some examples, a targeting moiety comprises a variable light chain (VL). In some examples, a targeting moiety comprises a Fab-like bispecific antibodies (bsFab). In some examples, a targeting moiety comprises a single-domain antibody-linked Fab (s-Fab). In some examples, a targeting moiety comprises an antibody or a fragment thereof.

[0074] In some examples, the targeting moiety comprises an antigen-binding moiety, wherein the antigen is expressed on an immune cell. In some examples, the targeting moiety specifically binds PD-1. In some examples, the targeting moiety

specifically binds PD-L1. In some examples, the targeting moiety specifically binds PD-L2. In some examples, the targeting moiety specifically binds CTLA- 4. In some examples, the targeting moiety specifically binds TIGIT. In some examples, the targeting moiety specifically binds TIM-3. In some examples, the targeting moiety specifically binds LAG-3. In some examples, the targeting moiety specifically binds CD25. In some examples, the targeting moiety specifically binds CD16a. In some examples, the targeting moiety specifically binds CD16b. In some examples, the targeting moiety specifically binds NKG2D. In some examples, the targeting moiety specifically binds NKP44. In some examples, the targeting moiety specifically binds NKP3. In some examples, the targeting moiety specifically binds CD19. In some examples, the targeting moiety specifically binds CD20. In some examples, the targeting moiety specifically binds CD30. In some examples, the targeting moiety specifically binds CD38. In some examples, the targeting moiety specifically binds BMCA. In some examples, the targeting moiety specifically binds HER2. In some examples, the targeting moiety specifically binds HER3. In some examples, the targeting moiety specifically binds DLL3. In some examples, the targeting moiety specifically binds DLL4. In some examples, the targeting moiety specifically binds EGFR. In some examples, the targeting moiety specifically binds GPC3. In some examples, the targeting moiety specifically binds c-MET. In some examples, the targeting moiety specifically binds VEGF-R1. In some examples, the targeting moiety specifically binds VEGF-R2. In some examples, the targeting moiety specifically binds OX40. In some examples, the targeting moiety specifically binds DR5. In some examples, the targeting moiety specifically binds ICOS. In some examples, the targeting moiety specifically binds GITR. In some examples, the targeting moiety specifically binds CD73. In some examples, the targeting moiety specifically binds CD39. In some examples, the targeting moiety specifically binds CD25. In some examples, the targeting moiety specifically binds CD16a. In some examples, the targeting moiety specifically binds CD8. In some examples, the targeting moiety specifically binds KLRC1. In some examples, the targeting moiety specifically binds KLRD1. In some examples, the targeting moiety specifically binds KLRB1. In some examples, the targeting moiety specifically binds CD40. In some examples, the

targeting moiety specifically binds CD137. In some examples, the targeting moiety specifically binds CD28.

[0075] In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding about 1-99% active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding about 5-80% active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding about 8-60% active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding about 10-40% active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding about 10-20% active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 3 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 5 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 8 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 10 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 12 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 15 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 20 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 25 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 30 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 35 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 40 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 45 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 50 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 60 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 70 % active cytokine. In some examples, the cleavable linker has an *in vitro* cleavage efficiency yielding at least 80 % active cytokine.

[0076] In one example, the present disclosure provides, among other things, a targeted cytokine comprising a targeting moiety, a cytokine or a fragment thereof, a masking moiety, and an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker, wherein the masking moiety binds to the cytokine or a fragment thereof, wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage, wherein the cleavable linker comprises MPYDLYHP (SEQ ID NO: 34) or VPLSLYSG (SEQ ID NO: 42) and wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

[0077] In one example, the present disclosure provides, among other things, a targeted cytokine comprising a targeting moiety, a cytokine or a fragment thereof, a masking moiety, and an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker, wherein the masking moiety binds to the cytokine or a fragment thereof, wherein the first or the second linker is a tumor specific cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage by a tumor specific protease, wherein the tumor specific cleavable linker comprises between 8-50 amino acid residues, and wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

[0078] In one aspect, the present disclosure provides, among other things, a targeted cytokine comprising a targeting moiety, a cytokine or a fragment thereof, a masking moiety, and an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker, wherein the masking moiety binds to the cytokine or a fragment thereof, wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage to yield active cytokine, wherein the cleavable linker has an *in vitro* cleavage efficiency yielding at least 10% active cytokine and wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

[0079] In one aspect, the present disclosure provides, among other things, a targeted cytokine comprising a targeting moiety, a cytokine or a fragment thereof, a masking moiety comprising CD122 or a fragment thereof comprising one or more mutations selected from the group consisting of F8C, A94C, L106C, C122S, C122V, C122A, N123C, N123Q, C168V, C168A, C168S, L169C, Q177C, V184C, S195C, R204C, and an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker, wherein the masking moiety binds to the cytokine or a fragment thereof, wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage, and wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

[0080] In another aspect, the present disclosure provides a targeted cytokine comprising: a) a targeting moiety, b) a masking moiety, c) a cytokine or fragment thereof, and d) a Fc domain comprising i. a first Fc polypeptide comprising a CH3 domain comprising a modification that reduces or eliminates binding to ***Protein A***, and ii. a second Fc polypeptide comprising a CH3 domain that binds to Protein A; and optionally, wherein the first Fc polypeptide is linked to the cytokine or a fragment thereof through a first linker and the second Fc polypeptide is linked to the masking moiety through a second linker; or wherein the first Fc polypeptide is linked to the masking moiety through a first linker and the second Fc polypeptide is linked to the cytokine or a fragment thereof through a second linker. In one example, the present disclosure provides, among other things, a targeted cytokine comprising a targeting moiety, a masking moiety, a cytokine or fragment thereof, and a Fc domain comprising a first Fc polypeptide comprising a CH3 domain comprising a modification that reduces or eliminates binding to Protein A, and a second Fc polypeptide comprising a CH3 domain that binds to Protein A. In another aspect, the present disclosure provides a targeted cytokine comprising: a) a ***targeting moiety that specifically binds to PD-1;*** b) a cytokine or a fragment thereof; c) a masking moiety; and d) an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker, wherein the masking moiety binds to the cytokine or a fragment thereof, wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage, wherein the ***cleavable linker comprises an amino acid sequence***

selected from Table 1 and wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

[0081] In one aspect, the present disclosure provides, among other things, a targeted cytokine comprising a targeting moiety, a cytokine or a fragment thereof, a masking moiety, and an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker, wherein the masking moiety binds to the cytokine or a fragment thereof, wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage, wherein the cleavable linker comprises MPYDLYHP (SEQ ID NO: 34) or VPLSLYSG (SEQ ID NO: 42) and wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

[0082] In one aspect, the present disclosure provides, among other things, a targeted cytokine comprising a targeting moiety that specifically binds to PD-1 comprising HCDR1 of SEQ ID NO: 4 (GYTFTNYY), HCDR2 of SEQ ID NO: 5 (INPSNGGT), HCDR3 SEQ ID NO: 6 (ARRDYRFDMGFDY), LCDR1 of SEQ ID NO: 9

(KGVSTSGYSY), LCDR2 of SEQ ID NO: 10 (LAS), LCDR3 of SEQ ID NO: 11 (QHSRDLPLT), a cytokine or fragment thereof, and an Fc domain. In another aspect, the present disclosure provides a targeted cytokine comprising: a) a targeting moiety that specifically binds to PD-1 comprising HCDR1 GITFSNSG (SEQ ID NO: 161), HCDR2 of VIWYDGSKRYYADSVKG (SEQ ID NO: 162), HCDR3 ATNDDY (SEQ ID NO: 163), LCDR1 QSVSSY (SEQ ID NO: 164), LDR2 DAS(SEQ ID NO: 165), and LCDR3 QQSSNWPRT(SEQ ID NO: 166), b) a cytokine or fragment thereof, and c) an Fc domain. In another aspect, the present disclosure provides a targeted cytokine comprising: a) a targeting moiety; b) an IL-2 cytokine or a fragment thereof; c) a VHH masking moiety; and d) an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker, wherein the masking moiety binds to the cytokine or a fragment thereof, wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage, wherein the VHH masking moiety comprises hCDR1 of SEQ ID NO: 169, hCDR of SEQ ID NO: 170, and hCDR3 of SEQ ID NO: 171, and wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

[0083] In one example, the present disclosure provides, among other things, a targeted IL-2 cytokine comprising SEQ ID NO: 87.

[0084] In some examples, the targeted IL-2 cytokine comprises SEQ ID NO: 88.

[0085] In some examples, the targeted IL-2 cytokine comprises SEQ ID NO: 89.

[0086] In some examples, the targeted IL-2 cytokine comprises SEQ ID NO: 90.

[0087] In some examples, the targeted IL-2 cytokine comprises SEQ ID NO: 91.

[0088] In one example, the present disclosure provides, among other things, a targeted IL-2 cytokine comprising SEQ ID NO: 87 and SEQ ID NO: 88, and a light chain comprising SEQ ID NO: 8.

[0089] In one example, the present disclosure provides, among other things, a targeted IL-2 cytokine comprising SEQ ID NO: 87 and SEQ ID NO: 89, and a light chain comprising SEQ ID NO: 8

[0090] In one example, the present disclosure provides, among other things, a targeted IL-2 cytokine comprising SEQ ID NO: 87 and SEQ ID NO: 90, and a light chain comprising SEQ ID NO: 8.

[0091] In one example, the present disclosure provides, among other things, a targeted IL-2 cytokine comprising SEQ ID NO: 87 and SEQ ID NO: 91, and a light chain comprising SEQ ID NO: 8.

[0092] In one aspect, the present disclosure provides, among other things, a nucleic acid encoding a target cytokine of the present disclosure. In one aspect, the present disclosure provides, among other things, a vector comprising a nucleic acid encoding a target cytokine of the present disclosure. In one example, the present disclosure provides,

among other things, a host cell comprising a nucleic acid encoding a target cytokine of the present disclosure. In another aspect, the present disclosure provides a host cell comprising the nucleic acid as described herein or the vector as described herein.

[0093] In one aspect, the present disclosure provides, among other things, a method of producing a targeted cytokine comprising culturing the host cell comprising a nucleic acid encoding a target cytokine of the present disclosure under a condition that produces the targeted cytokine.

[0094] In one aspect, the present disclosure provides, among other things, a composition comprising the targeted cytokine of the present disclosure.

[0095] In one aspect, the present disclosure provides, among other things, a pharmaceutical composition comprising the targeted cytokine of the present disclosure and a pharmaceutically acceptable carrier.

[0096] In one example, the present disclosure provides, among other things, a kit comprising the targeted cytokine of the present disclosure. In another aspect, the present disclosure provides a comprising the targeted cytokine as described herein, or the composition as described herein, or the pharmaceutical composition as described herein.

[0097] In another aspect, the present disclosure provides a use of the targeted cytokine as described herein, in the manufacture of a medicament a) for treating a neoplastic disease; b) for treating or preventing an inflammatory disease; or c) for treating or preventing an autoimmune disease. In another aspect, the present disclosure provides the targeted cytokine as described herein for use in therapy. In one example, the present disclosure provides, among other things, a method of treating or preventing a neoplastic disease in a subject, the method comprising administering to the subject an effective amount of the targeted cytokine of the present disclosure.

[0098] In one example, the present disclosure provides, among other things, a method of treating or preventing a neoplastic disease in a subject, the method comprising administering to the subject an effective amount of a composition comprising the targeted cytokine of the present disclosure.

[0099] In one example, the present disclosure provides, among other things, a method of treating or preventing a neoplastic disease in a subject, the method comprising

administering to the subject an effective amount of a pharmaceutical composition comprising the targeted cytokine of the present disclosure.

[0100] In one example, the present disclosure provides, among other things, a method of treating or preventing an inflammatory or autoimmune disease in a subject, the method comprising administering to the subject an effective amount of the targeted cytokine of the present disclosure

[0101] In one example, the present disclosure provides, among other things, a method of treating or preventing an inflammatory or autoimmune disease in a subject, the method comprising administering to the subject an effective amount of a composition comprising the targeted cytokine of the present disclosure.

[0102] In one example, the present disclosure provides, among other things, a method of treating or preventing an inflammatory or autoimmune disease in a subject, the method comprising administering to the subject an effective amount of a pharmaceutical composition comprising the targeted cytokine of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0103] The drawings are for illustration purposes only, not for limitation.

[0104] **FIG. 1** illustrates exemplary schematics of the Fc domains used in the targeted IL-2 cytokines of the present invention. The first Fc polypeptide comprises the CH2 and CH3 domains derived from IgG1. The second chain of the "chimeric Fc" comprise a CH2 domain derived from the IgG1, and a CH3 domain derived from IgG3. The second chain of the "CH3 mutant" comprises CH2 and CH3 domains derived from IgG1 with "RF mutations" (H435R and Y436F) in the CH3 domain.

[0105] **FIG. 2A** is series of exemplary SDS-PAGE analysis of purified masked IL-2 cytokines comprising either the native Fc, CH3 mutant, or chimeric Fc domain. Each construct was purified by Protein A chromatography and size exclusion chromatography (SEC) at different ratios of the first and second Fc polypeptides. **FIG. 2B** is a series of exemplary EC50 graphs of purified masked IL-2 cytokines comprising either the native Fc, CH3 mutant, or chimeric Fc domain. EC50 values were determined in a cell-based reporter assay (HEK Blue IL-2 assay) which measures % calculated active cytokine.

[0106] **FIG. 3** illustrates exemplary schematics of the targeted IL-2 cytokine (TC) molecules of the present invention, and their purities as determined by SEC, non-reducing capillary electrophoresis SDS (CE-SDS), and reducing CE-SDS). The TC molecules tested in this experiment all comprise bivalent Fabs that bind to PD-1, a first Fc polypeptide with "hole mutations" (Y349C; T366S; L368A; and Y407V), a second Fc polypeptide with "knob mutations" (S354C and T366W) fused to an IL-2 cytokine. TC1 is "unmasked," without a masking moiety that binds to IL-2. TC2, TC3, and TC4

CLAIMS

1. A targeted cytokine comprising:

a) a targeting moiety;

b) a cytokine or a fragment thereof;

c) a masking moiety; and

d) an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker,

wherein the masking moiety binds to the cytokine or a fragment thereof,

wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage,

wherein the cleavable linker comprises (i) ***MPYDLYHP (SEQ ID NO: 34) or VPLSLYSG (SEQ ID NO: 42),*** (ii) ***an amino acid sequence selected from Table 1, or (iii) between 8-50 amino acid residues*** and

wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

2. A targeted cytokine comprising:

a) a targeting moiety;

b) a cytokine or a fragment thereof;

c) a masking moiety; and

d) an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker;

wherein the masking moiety binds to the cytokine or a fragment thereof,

wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage to yield active cytokine,

wherein the cleavable linker has an ***in vitro cleavage efficiency yielding at least 10% active cytokine*** and

wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

3. A targeted cytokine comprising:

a) a targeting moiety;

b) a cytokine or a fragment thereof;

c) a masking moiety comprising ***CD122 or a fragment thereof comprising one or more mutations selected from the group consisting of F8C, A94C, L106C, C122S, C122V, C122A, N123C, N123Q, C168V, C168A, C168S, L169C, Q177C, V184C, S195C, R204C***; and

d) an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker,

wherein the masking moiety binds to the cytokine or a fragment thereof,

wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage, and wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

4. A targeted cytokine comprising:

a) a targeting moiety,

b) a masking moiety,

c) a cytokine or fragment thereof, and

d) a Fc domain comprising

i. a first Fc polypeptide comprising a CH3 domain comprising a modification that reduces or eliminates binding to ***Protein A***, and

ii. a second Fc polypeptide comprising a CH3 domain that binds to Protein A; and optionally,

wherein the first Fc polypeptide is linked to the cytokine or a fragment thereof through a first linker and the second Fc polypeptide is linked to the masking moiety through a second linker; or

wherein the first Fc polypeptide is linked to the masking moiety through a first linker and the second Fc polypeptide is linked to the cytokine or a fragment thereof through a second linker.

5. The targeted cytokine of claim 4, wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

6. The targeted cytokine of any one of claims 1-5, wherein the targeting moiety specifically binds PD-1, PD-L1, PD-L2, CTLA-4, TIGIT, TIM-3, LAG-3, CD25, CD16a, CD16b, NKG2D, NKP44, NKP30, CD19, CD20, CD30, CD38, BCMA, human epidermal growth factor receptor 2 (HER2), human epidermal growth factor receptor 3 (HER3), delta-like protein 3 (DLL3), delta-like protein 4 (DLL4), epidermal growth factor receptor (EGFR), glypican-3 (GPC3), c-MET, vascular endothelial growth factor receptor 1 (VEGF R1), vascular endothelial growth factor receptor 2 (VEGF R2), Nectin-4, Liv-1, glycoprotein NMB (GPNMB), prostate specific membrane antigen (PSMA), Trop-2, carbonic anhydrase IX (CA9), endothelin B receptor (ETBR), six transmembrane epithelial antigen of the prostate 1 (STEAP1), folate receptor alpha (FR-a), SLIT and NTRK-like protein 6 (SLITRK6), carbonic anhydrase VI (CA6), ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3), mesothelin, trophoblast glycoprotein (TPBG), CD19, CD20, CD22, CD33, CD40, CD56, CD66e, CD70, CD74, CD79b, CD98, CD 123, CD 138, CD352, CD47, signal-regulatory protein alpha (SIRPα), Claudin 18.2, Claudin 6, 5T4, fibroblast activation protein alpha (FAPα), the melanoma-associated chondroitin sulfate proteoglycan (MCSP), epithelial cellular adhesion molecule (EPCAM), or combinations thereof.

7. A targeted cytokine comprising:
   a) a targeting moiety that specifically binds to ***PD-1;***
   b) a cytokine or a fragment thereof;
   c) a masking moiety; and

d) an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker,

wherein the masking moiety binds to the cytokine or a fragment thereof,

wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage,

wherein the cleavable linker comprises ***MPYDLYHP (SEQ ID NO: 34) or VPLSLYSG (SEQ ID NO: 42)*** and

wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

8. A targeted cytokine comprising:

a) a ***targeting moiety that specifically binds to PD-1;***

b) a cytokine or a fragment thereof;

c) a masking moiety; and

d) an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker,

wherein the masking moiety binds to the cytokine or a fragment thereof,

wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage,

wherein the ***cleavable linker comprises an amino acid sequence selected from Table 1*** and

wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

9. A targeted cytokine comprising:

a) a targeting moiety that specifically binds to PD-1 comprising ***HCDR1 of SEQ ID NO: 4 (GYTFTNYY), HCDR2 of SEQ ID NO: 5 (INPSNGGT), HCDR3 SEQ ID NO: 6 (ARRDYRFDMGFDY), LCDR1 of SEQ ID NO: 9 (KGVSTSGYSY),***

LCDR2 of SEQ ID NO: 10 (LAS), and LCDR3 of SEQ ID NO: 11 (QHSRDLPLT);

b) a cytokine or fragment thereof, and

c) an Fc domain.

10. A targeted cytokine comprising:

a) a targeting moiety that specifically binds to PD-1 comprising HCDR1 GITFSNSG (SEQ ID NO: 161), HCDR2 of VIWYDGSKRYYADSVKG (SEQ ID NO: 162), HCDR3 ATNDDY (SEQ ID NO: 163), LCDR1 QSVSSY (SEQ ID NO: 164), LDR2 DAS(SEQ ID NO: 165), and LCDR3 QQSSNWPRT(SEQ ID NO: 166).

b) a cytokine or fragment thereof, and

c) an Fc domain.

11. The targeted cytokine of any one of claims 9 or 10, wherein the Fc domain comprises

i. a first Fc polypeptide comprising a CH3 domain comprising a modification that reduces or eliminates binding to Protein A, and

ii. a second Fc polypeptide comprising a CH3 domain that binds to Protein A.

12. A targeted cytokine comprising:

a) a targeting moiety***;***

b) an IL-2 cytokine or a fragment thereof;

c) a VHH masking moiety; and

d) an Fc domain comprising a first Fc polypeptide linked to the cytokine or a fragment thereof through a first linker and a second Fc polypeptide linked to the masking moiety through a second linker,

wherein the masking moiety binds to the cytokine or a fragment thereof,

wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage,

wherein the VHH masking moiety comprises hCDR1 of SEQ ID NO: 169, hCDR of SEQ ID NO: 170, and hCDR3 of SEQ ID NO: 171, and

wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

13. The targeted cytokine of any one of claims 9-12, wherein the first Fc polypeptide is linked to the cytokine or a fragment thereof through a first linker and the second Fc polypeptide is linked to the masking moiety through a second linker, optionally wherein the targeting moiety is linked to the Fc domain through one or both of the first and second Fc polypeptides.

14. The targeted cytokine of any one of claims 9-13, wherein the masking moiety binds to the cytokine or a fragment thereof and/or wherein the first or the second linker is a cleavable linker such that the masking moiety releases the cytokine or a fragment thereof upon cleavage.

15. The targeted cytokine of any one of claims 9-14, wherein the cleavable linker comprises MPYDLYHP (SEQ ID NO: 34), VPLSLYSG (SEQ ID NO: 42), PVSLRSGS (SEQ ID NO: 196), GMPKDLYHAS (SEQ ID NO. 197), RPLALWRS (SEQ ID NO: 193), TQKPLGLS (SEQ ID NO:194), APAGLIVPYN (SEQ ID NO:195), PANLVAPDP (SEQ ID NO: 183), IVGRPRHQGV (SEQ ID NO:199), RSKYLATA (SEQ ID NO:198), or RAAAVKSP (SEQ ID NO:37).

16. The targeted cytokine of any one of the preceding claims, wherein the cleavable linker comprises a proteolytically cleavable peptide flanked on both sides by a spacer domain, optionally wherein the spacer domain is between 3 and 10 amino acids or between 3 and 6 amino acids in length.

17. The targeted cytokine of any one of the preceding claims, wherein the first linker is the cleavable linker or the second linker is the cleavable linker.

18. The targeted cytokine of any one of the preceding claims, wherein (i) the C-terminus of the targeting moiety is linked to the N-terminus of the first Fc polypeptide, (ii) the C-terminus of the first Fc polypeptide is linked to the N-terminus of the cytokine or a

fragment thereof, (iii) the C-terminus of the targeting moiety is linked to the N-terminus of the second Fc polypeptide, and/or (iv) the C-terminus of the second Fc polypeptide is linked to the N-terminus of the masking moiety.

19. The targeted cytokine of any one of the preceding claims, wherein the targeting moiety and/or a masking moiety comprises one or more antigen binding domains, a peptide, a polypeptide, a protein, a ligand, or an agent that specifically binds to the cytokine or the fragment thereof, or wherein the targeting moiety comprises an antigen binding domain selected from the group consisting of Fab, a single chain Fv (scFv), a single domain antibody (VHH), one or more CDRs, a variable heavy chain (VH), a variable light chain (VL), a Fab-like bispecific antibodies (bsFab), a single-domain antibody-linked Fab (s-Fab), an antibody, and a combination thereof.

20. The targeted cytokine of any one of the preceding claims, wherein the targeted cytokine comprises a first antigen binding domain and a second antigen binding domain, optionally wherein (i) the first and second antigen binding domains specifically bind to the same target, (ii) the first and second antigen binding domains comprise the same amino acid sequence, (iii) the first and second antigen binding domains specifically bind to different targets, or (iv) the first and second antigen binding domains comprise different amino acid sequences.

21. The targeted cytokine of claim 20,

wherein the C-terminus of the first antigen binding domain is linked to the N-terminus of the first Fc polypeptide;

wherein the C-terminus of the first Fc polypeptide is linked to the N-terminus of the cytokine or a fragment thereof;

wherein the C-terminus of the second antigen binding domain is linked to the N-terminus of the second Fc polypeptide; and

wherein the C-terminus of the second Fc polypeptide is linked to the N-terminus of the masking moiety.

22. The targeted cytokine of any one of the preceding claims, wherein the first Fc polypeptide and/or the second Fc polypeptide comprise a first IgG1, IgG2, or IgG4 Fc domain or a fragment thereof.

23. The targeted cytokine of claim any one of the preceding claims, wherein the first and/ or the second Fc domains each contain one or more modifications that promote the non-covalent association of the first and the second Fc polypeptide, optionally, wherein

a) the first Fc domain comprises Y349C, T366S, L368A, Y407V, and N297A mutations and the second Fc domain comprises S354C, T366W and N297A mutations; or
b) the second Fc domain comprises Y349C, T366S, L368A, Y407V, and N297A mutations and the first Fc domain comprises S354C, T366W and N297A mutations;
c) the first Fc domain comprises Y349C, T366S, L368A, Y407V, N297A and I253A mutations and the second Fc domain comprises S354C, T366W, N297A and I253A mutations; or
d) the second Fc domain comprises Y349C, T366S, L368A, Y407V, N297A and I253A mutations and the first Fc domain comprises S354C, T366W, N297A and I253A mutations; or
e) wherein the first Fc polypeptide comprises a first CH3 domain comprising a modification that reduces or eliminates binding to Protein A and the second Fc polypeptide comprises a second CH3 domain that binds to Protein A.

24. The targeted cytokine of claim 23, wherein the CH3 domain that binds to Protein A is a human IgG1, IgG2 or IgG4 sequence, optionally wherein the second CH3 domain is a human IgG1, IgG2 or IgG4 sequence comprising a modification at position H435 and/or Y436 according to Kabat numbering.

25. The targeted cytokine of any one of the preceding claims, wherein (i) the first or second Fc domain comprises SEQ ID NO: 85 or SEQ ID NO: 80, or (ii) the first or second Fc domain comprises SEQ ID NO: 84 or SEQ ID NO: 80.

26. The targeted cytokine of any one of the preceding claims, wherein the cytokine or a fragment thereof is IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-20, TNF-α, TNF-β, CXCL8 (IL-18), G-CSF, GM-CSF, LIF, OSM, IFN-α, IFN-β, IFN-γ, CD154, LT-β, 4-1BBL, APRIL, CD70, CD153, CD178, GITRL, LIGHT, OX40L, TALL-1, TRAIL, TWEAK, TRANCE, TGF-β, M-CSF, or MSP or a fragment thereof.

27. The targeted cytokine of any one of claims 1-2, 4-11, or 13-26, wherein the masking moiety is a receptor of the cytokine or the fragment thereof, optionally, CD132, CD124, CD213a13, CD132, CD127, IL-9R, CD213a1, CD213a2, CD1243, CD132, IL-15Ra, CDw131, CDw125, CD131, CD116, CD126, CD130, IL-11Ra, CD114, CD212, LIFR, OSMR, IL-20Rα, IL-20Rβ, IL-14R, CD4, CDw127, CD118, CDw119, CD40, LTβR, CD120a, CD120b, CDw137, BCMA, TACI, CD27, CD30, CD95, GITR, LTbR, HVEM, OX40, TRAILR1-4, Apo3, RANK, OPG, TGF-βR1, TGF-βR2, TGF-βR3, CD115, or CDw136.

28. The targeted cytokine of claim 3 or 27, wherein the masking moiety is an engineered CD122 comprising mutations selected from

a) C122S and C168S,
b) C122V and C168V,
c) C122A and C168V,
d) C168V,
e) C122V and C168A,
f) C122A and N123C,
g) C122V and N123C,
h) C122A and C168A,

i) V117C, N123Q and C168A,
j) N123Q, C168A, and L169C,
k) L106C, C122A, C168A and S195C
l) L106C, C122A, C168A and V184C
m) C122A, C168A, V184C, and S195C,
n) C122A, C168A, Q177C, and R204C,
o) L106C, C122V, C168V, and S195C or
p) F8C, A94C, C122V, and C168V.

29. The targeted cytokine of any one of the preceding claims, wherein the cytokine is a modified IL-2 cytokine or functional fragment thereof compared to the sequence of a mature IL-2 having SEQ ID NO: 13.

30. The targeted cytokine of claim 29, wherein the modified IL-2 cytokine or functional fragment thereof comprises modifications that (i) reduce or eliminate binding to CD25, (ii) enhance binding to CD25, (iii) reduce or eliminate binding to CD122, or (iv) enhance binding to CD122,

optionally, wherein the modified IL-2 cytokine or functional fragment thereof comprises (i) modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 13, (ii) modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 13, (iii) R38A, F42A, Y45A, E62A and C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 13, or (iv) an amino acid sequence of SEQ ID NO: 14.

31. The targeted cytokine of any one of claims 1-11 or 13-28, wherein the cytokine comprises an IL-15 polypeptide comprising an amino acid sequence of SEQ ID NO: 93 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 93, optionally wherein the modification comprises (i) one or more amino acid substitutions at positions D22, E46, or E53 as compared to the amino acid sequence of SEQ ID NO: 93, (ii) one or more amino acid substitutions at positions D22, E46, E53, N71, N79, or N112 as

compared to the amino acid sequence of SEQ ID NO: 93, (iii) amino acid substitutions at positions N71 and N79 of SEQ ID NO: 93, (iv) amino acid substitutions at positions N71 and N112 of SEQ ID NO: 93, or (v)) amino acid substitutions at positions N79 and N112 of SEQ ID NO: 93.

32. The targeted cytokine of any one of claims 1-11 or 13-28, wherein the cytokine comprises an IL-12 polypeptide or functional fragment thereof comprising an IL-12p40 polypeptide or functional fragment thereof covalently linked to an IL-12p35 polypeptide or functional fragment thereof.

33. The targeted cytokine of claim 32, wherein the IL-12p40 – IL-12p35 linker is between 5 and 20 amino acids in length, optionally wherein the IL-12p40 – IL-12p35 linker (i) is rich in amino acid residues G and S, or (ii) comprises SEQ ID NO: 119 (GGGGSGGGGSGGGGS).

34. The targeted cytokine of any one of claims 32-33, wherein the IL-12p40 polypeptide comprises SEQ ID NO: 111 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 111, optionally wherein IL-12p40 polypeptide comprises at least one amino acid modification to the GAG-binding domain of SEQ ID NO: 114 (KSKREKKDRV) as compared to the amino acid sequence of SEQ ID NO: 111.

35. The targeted cytokine of any one of claims 32-34, wherein IL-12p40 polypeptide comprises SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, or SEQ ID NO: 118.

36. The targeted cytokine of any one of claims 32-35, wherein the IL-12p35 polypeptide comprises SEQ ID NO: 112 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 11.

37. The targeted cytokine of any one of claims 32-36, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, or SEQ ID NO: 154.

38. The targeted cytokine of any one of claims 32-37, wherein the masking moiety comprises the extracellular domain of human IL-12Rβ1 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12, optionally, masking moiety comprises (i) a sequence having SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132 or SEQ ID NO: 133; or (ii) the extracellular domain of human IL-12Rβ2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.

39. A nucleic acid encoding the targeted cytokine of any one of the preceding claims.

40. A vector comprising the nucleic acid of claim 39.

41. A host cell comprising the nucleic acid of claim 39 or the vector of claim 40.

42. A method of producing a targeted cytokine comprising culturing the host cell of claim 41 under a condition that produces the targeted cytokine.

43. A composition comprising the targeted cytokine of any one of claims 1-38.

44. A pharmaceutical composition comprising the targeted cytokine of any one of claims 1-38, and a pharmaceutically acceptable carrier.

45. A kit comprising the targeted cytokine of any one of claims 1-38, or the composition of claim 43, or the pharmaceutical composition of claim 44.

46. Use of the targeted cytokine of any one of claims 1-38, in the manufacture of a medicament

a) for treating a neoplastic disease;

b) for treating or preventing an inflammatory disease; or

c) for treating or preventing an autoimmune disease.

47. The targeted cytokine of any one of claims 1-38 for use in therapy.