not shared by other STAT3 activating cytokine receptors. Riley, et al. (1999) Journal of Biological Chemistry 274(23):15967-16664.

[0012] The expression of the hIL10Rα and IL10Rb receptor subunits vary with respect to cell type and the activation state of the cell. The activation state of cells that express hIL10Rα may result in substantial variability in the levels of expression. The expression hIL10Rα in hematopoietic cells, which constitutively express low levels of hIL10Rα, is frequently substantially upregulated by various stimuli. In contrast to hIL10Rα, which is expressed primarily on hematopoietic cells, the IL10Rb receptor subunit is expressed ubiquitously. While certain cell types express hIL10Rβ at different levels, the level of hIL10Rβ expression in a given cell type is typically less affected by the activation state of the cell than hIL10Rα.

[0013] hIL10 is associated with a wide variety of functions and exhibits both immunosuppressive and immunostimulatory activities through its interaction with T cells, B cells, macrophages, and antigen presenting cells (APCs). The immunosuppressive activity of hIL10 is well documented. hIL10 is associated with suppressing the expression of IL1α, IL1β, IL6, IL8, TNFα, GM-CSF and G-CSF in activated monocytes and activated macrophages, as well as suppression of proinflammatory cytokine interferon-gamma (INFγ) production by NK cells. However, hIL10 also demonstrates an immunostimulatory effect by stimulation of the production of the proinflammatory cytokine IFN-γ by CD8+ T cells. The immunostimulatory and immunosuppressive properties of hIL10 have proved to be a challenge in the clinical application of hIL10 in the treatment of human disease.

[0014] Saxton, *et al*. describe the interaction of IL10 with the IL10Rb subunit and amino acid residues that are involved in the binding of IL10 to IL10Rβ and that modification of such residues can potentially provide IL10 variants that retain the immunosuppressive function of IL10 on myeloid cells but have diminished pro-inflammatory activity on CD8+ T cells. Saxton, *et al*. (2021) Science 371 (6535), eabc8433.

SUMMARY

[0015] The present disclosure provides compositions that are useful for modulating signal transduction mediated by interleukin-10 (IL10). In particular, the disclosure provides inverted monomers and dimers thereof that are partial agonists of STAT3-mediated signaling (“STAT3 signaling”). In some examples, the inverted monomers and dimers thereof described herein activate STAT3 signaling in some cell types, and result in decreased STAT3 signaling in other cell types. In some examples, the inverted monomers and dimers thereof described herein

activate STAT3 signaling in myeloid cells, and have decreased STAT3 signaling in lymphocytes. In some examples, the inverted monomers and dimers thereof described herein demonstrated a preferential activation of STAT3 signaling in myeloid cells relative to lymphocytes as compared to wt hIL10.

[0016] In one aspect, provided herein is a polypeptide comprising an amino acid sequence of the formula 1:

[A]-L1x-[B]-L2-[C]-L3-[D]-L4y-[E]-L5-[F] (1)

wherein:

x and y are independently selected from 0 (absent) or 1 (present);

[A] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence SKAVEQVKNAFNKL (SEQ ID NO:1);

x = 1 and L1 comprises a polypeptide linker of from 1 to 5 amino acids, or x = 0 (no linker, L1 is absent);

[B] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence “EKGIYKAMSEFDIFINYIEAYMTMKIR” (SEQ ID NO:2);

L2 comprises a linker of from 10 to 25 amino acids;

[C] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence “NLPNMLRDLRDAFSRVKTFFQMKD” (SEQ ID NO:3);

L3 comprises a linker of from 4 to 11 amino acids;

[D] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence “KESLLEDFKG” (SEQ ID NO:4);

y = 1 and L4 comprises a polypeptide linker of from 1 to 5 amino acids, or y = 0 (L4 is absent);

[E] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence “LGCQALSEMIQFYLEEVMPQAEN” (SEQ ID NO:5);

L5 comprises a linker of from 1 to 7 amino acids; and

[F] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence “IKAHVNSLGENLKTLRLRLRRC” (SEQ ID NO:6),

optionally wherein L1, L2, L3, L4 and/or L5 comprise a GS-linker,

optionally wherein L1 is the amino acid glutamine (Q),

optionally wherein L2 is a polypeptide having the amino acid sequence NSPGQGTQSENSCTHFPG (SEQ ID NO:7) or NTSPGQGTQSENSCTHFPG (SEQ ID NO:23),

optionally wherein L3 is a polypeptide having the amino acid sequence QLDNLLL (SEQ ID NO:8),

optionally wherein L4 is the amino acid glycine ("G") or comprises the amino acid sequence GY,

optionally wherein L5 is a polypeptide having the amino acid sequence QDPD (SEQ ID NO:9),

wherein optionally the polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity hIL10 inverted monomer selected from the group consisting of SEQ ID NO: 10, SEQ ID NO:11, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, and SEQ ID NO:98, or

optionally wherein the polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:10 or SEQ ID NO:11.

[0016a] In one example a polypeptide is provided comprising an amino acid sequence of the formula 1:

[A]-L1x-[B]-L2-[C]-L3-[D]-L4y-[E]-L5-[F] (1)

wherein:

x and y are independently selected from 0 (absent) or 1 (present);

[A] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence SKAVEQVKNAFNKL (SEQ ID NO:1);

x = 0 (no linker, L1 is absent). or

x = 1 and L1 comprises a polypeptide linker of from 1 to 5 amino acids;

[B] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence "EKGIYKAMSEFDIFINYIEAYMTMKIR" (SEQ ID NO:2);

L2 comprises a linker of from 10 to 25 amino acids;

[C] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence “NLPNMLRDLRDAFSRVKTFFQMKD” (SEQ ID NO:3);

L3 comprises a linker of from 4 to 11 amino acids;

[D] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence “KESLLEDFKG” (SEQ ID NO:4);

y = 0 (L4 is absent), or

y = 1 and L4 comprises a polypeptide linker of from 1 to 5 amino acids;

[E] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence “LGCQALSEMIQFYLEEVMPQAEN” (SEQ ID NO:5);

L5 comprises a linker of from 1 to 7 amino acids; and

[F] is a polypeptide having 0, 1 or 2 amino acid substitutions relative to the amino acid sequence “IKAHVNSLGENLKTLRLRLRRC” (SEQ ID NO:6).

[0017] In some examples, L1, L2, L3, L4 and/or L5 comprise a GS-linker. In some examples, L1 is the amino acid glutamine (Q). In some examples, L2 is a polypeptide having the amino acid sequence NSPGQGTQSENSCTHFPG (SEQ ID NO:7) or NTSPGQGTQSENSCTHFPG (SEQ ID NO:23). In some examples, L3 is a polypeptide having the amino acid sequence QLDNLLL (SEQ ID NO:8). In some examples, L4 is the amino acid glycine ("G") or comprises the amino acid sequence GY. In some examples, L5 is a polypeptide having the amino acid sequence QDPD (SEQ ID NO:9). In some examples, the polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:10 or SEQ ID NO:11.

[0018] In some examples, the polypeptide comprises the amino acid sequence

KSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRNSPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRCHRFLPCEN (SEQ ID NO:10), or

[0019] In some examples, the polypeptide comprises the amino acid sequence

KSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRNTSPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRCHRFLPCEN (SEQ ID NO:11), or an amino acid sequence having at least 95% sequence identity to SEQ ID NO:10 or SEQ ID NO: 11.

[0020] In some examples, the polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity hIL10 inverted monomer selected from the group consisting of SEQ ID NO: 10, SEQ ID NO:11, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, and SEQ ID NO:98

[0021] In some examples, a polypeptide is provided comprising formula (2):

$$[\text{Monomer 1}]\text{-linker}_x\text{-}[\text{Monomer 2}] \qquad (2)$$

wherein Monomer 1 and Monomer 2 are polypeptides of formula 1 and Monomer 1 and Monomer 2 are the same or different, and x = 0 (linker absent) or 1 (linker present).

[0022] In some examples, the polypeptide of formula (2) comprises in the following amino to carboxyl order:

(a) Monomer 1 comprising:

(i) a first polypeptide comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a polypeptide selected from the group consisting amino acid residues 117-158 of human IL-10 (SEQ ID NO:12); amino acid residues 117-159 of human IL-10 (SEQ ID NO:13); and amino acid residues 117-160 of human IL-10 (SEQ ID NO:14), numbered in accordance with mature wild-type human IL-10 (SEQ ID NO:20);

(ii) a first $linker_x$, wherein x = 0 (linker absent) or 1 (linker present); and

(iii) a second polypeptide comprising an amino acid having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a polypeptide sequence selected from the group consisting of amino acid residues 1-160 of human IL-10 (SEQ ID NO:20); amino acid residues 2-160 of human IL-10 (SEQ ID NO:36); amino acid residues 3-160 of human IL-10 (SEQ ID NO:37); amino acid residues 4-160 of human IL-10 (SEQ ID NO:38); amino acid residues 5-160 of human IL-10 (SEQ ID NO:39); amino acid residues 6-160 of human IL-10 (SEQ ID NO:40); amino acid residues 7-160 of human IL-10 (SEQ ID NO:41); amino acid residues 8-160 of human IL-10 (SEQ ID NO:42); amino acid residues 9-160 of human IL-10 (SEQ ID NO:43); amino acid residues 10-160 of human IL-10 (SEQ ID NO:44); and amino acid residues 11-160 of human IL-10 (SEQ ID NO:45) numbered in accordance with mature wild-type hIL10 (SEQ ID NO:20);

(b) a second $linker_y$, wherein y = 0 (linker absent) or 1 (linker present); and

(c) Monomer 2 comprising a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a polypeptide sequence selected from the group consisting of amino acid residues 1-116 of human IL-10 (SEQ ID NO:15); amino acid residues 2-116 of human IL-10 (SEQ ID NO:16); amino acid residues 3-116 of human IL-10 (SEQ ID NO:17), amino acid residues 4-116 of human IL-10 (SEQ ID NO:18), amino acid residues 5-116 of human IL-10 (SEQ ID NO:19), amino acid residues 6-116 of human IL-10 (SEQ ID NO:29), amino acid residues 7-116 of human IL-10 (SEQ ID NO:30), amino acid residues 8-116 of human IL-10 (SEQ ID NO:31), amino acid residues 9-116 of human IL-10 (SEQ ID NO:32), amino acid residues 10-116 of human IL-10 (SEQ ID NO:33); and amino acid residues 11-116 of

human IL-10 (SEQ ID NO:34), numbered in accordance with mature human IL-10 (SEQ ID NO:20).

[0023] In some examples, the polypeptide of formula (2) comprises in the following amino to carboxyl order:

(a) Monomer 1 comprising:

(i) a first polypeptide comprising an amino acid sequence selected from the group consisting amino acid residues 117-158 of human IL-10 (SEQ ID NO:12); amino acid residues 117-159 of human IL-10 (SEQ ID NO:13); and amino acid residues 117-160 of human IL-10 (SEQ ID NO:14), numbered in accordance with mature human IL-10 (SEQ ID NO:20),

(ii) a first $linker_x$;

(iii) a second polypeptide comprising an amino acid sequence selected from the group consisting of amino acid residues 1-116 of human IL-10 (SEQ ID NO:15);

(b) a second $linker_y$;

(c) Monomer 2 comprising:

(i) a first polypeptide comprising an amino acid sequence selected from the group consisting amino acid residues 117-158 of human IL-10 (SEQ ID NO:12); amino acid residues 117-159 of human IL-10 (SEQ ID NO:13); and amino acid residues 117-160 of human IL-10 (SEQ ID NO:14), numbered in accordance with mature human IL-10 (SEQ ID NO:20),

(ii) a third $linker_z$; and

(iii) a second polypeptide comprising an amino acid sequence selected from the group consisting of amino acid residues 1-116 of human IL-10 (SEQ ID NO:15).

[0024] In some examples, the polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity hIL10 inverted monomer selected from the group consisting of SEQ ID NOS:46-51.

[0025] In some examples, the polypeptide exhibits cell type biased activity relative to the wild type IL10 species from which the inverted IL10 monomer was derived. In some

examples, the polypeptide (a) exhibits a significant level of at least one anti-inflammatory property of wild type IL10 and (b) exhibits a significantly reduced level of at least one pro-inflammatory property of wild type IL10. In some examples, the at least one anti-inflammatory property is selected from the group consisting of suppression of expression or secretion of IL1β, TNFα or IL6 in a myeloid cell. In some examples, the at least one pro-inflammatory property is selected from the group consisting of suppression of expression or secretion of IFNγ, granzyme A or granzyme B in a T cell. In some examples, the at least one anti-inflammatory property is selected from the group consisting of suppression of expression or secretion of IL1β, TNFα or IL6 in a myeloid cell and the at least one pro-inflammatory property is selected from the group consisting of suppression of expression or secretion of IFNγ, granzyme A or granzyme B in a T cell.

[0026] In some examples, the cell type biased activity is the production of phospho-STAT3. In some examples, wherein the pSTAT3 Emax of the polypeptide is greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, or greater than 70% of the pSTAT3 Emax of wild-type hIL10 in myeloid cells.

[0027] In some examples, the pSTAT3 Emax in a lymphocyte less than 70%, less than 60%, less than 50%, less than 40%, or less than 30%, of the pSTAT3 Emax of a wild-type hIL10 in the lymphocyte.

[0028] In some examples, (a) the pSTAT3 Emax of the polypeptide is greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, or greater than 70% of the pSTAT3 Emax of wild-type hIL10 in myeloid cells and (b) the pSTAT3 Emax in a lymphocyte less than 70%, less than 60%, less than 50%, less than 40%, or less than 30%, of the pSTAT3 Emax of a wild-type hIL10 in the lymphocyte.

[0029] In another aspect, provided herein is a polypeptide comprising formula (2):

$$[\text{Monomer 1}]\text{-linker}_x\text{-}[\text{Monomer 2}] \qquad (2)$$

wherein Monomer 1 and Monomer 2 each, independently, comprise a polypeptide selected from a polypeptide of claim 1, and x = 0 (linker absent) or 1 (linker present),

wherein optionally Monomer 1 and Monomer 2 each, independently, comprise a polypeptide comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to an amino acid sequence selected from

the group consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO: 90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, and SEQ ID NO:98, optionally wherein x is 1 (linker present) and the linker comprises a GS-linker.

[0029a] In some examples, a polypeptide is provided comprising formula (2):

[Monomer 1]-linker$_x$-[Monomer 2] (2)

wherein Monomer 1 and Monomer 2 each, independently, comprise a polypeptide selected from a polypeptide of claim 1, and x = 0 (linker absent) or 1 (linker present).

[0030] In some examples, Monomer 1 and Monomer 2 each, independently, comprise a polypeptide comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO: 90, SEQ ID NO:91, SEQ

ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, and SEQ ID NO:98.

[0031] In some examples, Monomer 1 and Monomer 2 each comprise a polypeptide comprising the amino acid sequence of SEQ ID NO:10.

[0032] In some examples, Monomer 1 and Monomer 2 each comprise a polypeptide comprising the amino acid sequence of SEQ ID NO:11.

[0033] In some examples, x is 1 (linker present) and the linker comprises a GS-linker.

[0034] In some examples, x is 0 (linker absent).

[0035] In some examples, the polypeptide of formula (2) comprises in the following amino to carboxyl order:

(a) Monomer 1 comprising:

(i) a first polypeptide comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a polypeptide selected from the group consisting amino acid residues 117-158 of human IL-10 (SEQ ID NO:12); amino acid residues 117-159 of human IL-10 (SEQ ID NO:13); and amino acid residues 117-160 of human IL-10 (SEQ ID NO:14), numbered in accordance with mature wild-type human IL-10 (SEQ ID NO:20);

(ii) a first $linker_x$, wherein x = 0 (linker absent) or 1 (linker present); and

(iii) a second polypeptide comprising an amino acid having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a polypeptide sequence selected from the group consisting of amino acid residues 1-160 of human IL-10 (SEQ ID NO:20); amino acid residues 2-160 of human IL-10 (SEQ ID NO:36); amino acid residues 3-160 of human IL-10 (SEQ ID NO:37); amino acid residues 4-160 of human IL-10 (SEQ ID NO:38); amino acid residues 5-160 of human IL-10 (SEQ ID NO:39); amino acid residues 6-160 of human IL-10 (SEQ ID NO:40); amino acid residues 7-160 of human IL-10 (SEQ ID NO:41); amino acid residues 8-160 of human IL-10 (SEQ ID NO:42); amino acid residues 9-160 of human IL-10 (SEQ ID NO:43); amino acid residues 10-160 of human IL-10 (SEQ ID NO:44); and amino acid residues 11-160 of human IL-10 (SEQ ID NO:45) numbered in accordance with mature wild-type hIL10 (SEQ ID NO:20);

(b) a second $linker_y$, wherein y = 0 (linker absent) or 1 (linker present); and

(c) Monomer 2 comprising a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a polypeptide sequence selected from the group consisting of amino acid residues 1-116 of human IL-10 (SEQ ID NO:15); amino acid residues 2-116 of human IL-10 (SEQ ID NO:16); amino acid residues 3-116 of human IL-10 (SEQ ID NO:17), amino acid residues 4-116 of human IL-10 (SEQ ID NO:18), amino acid residues 5-116 of human IL-10 (SEQ ID NO:19), amino acid residues 6-116 of human IL-10 (SEQ ID NO:29), amino acid residues 7-116 of human IL-10 (SEQ ID NO:30), amino acid residues 8-116 of human IL-10 (SEQ ID NO:31), amino acid residues 9-116 of human IL-10 (SEQ ID NO:32), amino acid residues 10-116 of human IL-10 (SEQ ID NO:33); and amino acid residues 11-116 of human IL-10 (SEQ ID NO:34), numbered in accordance with mature human IL-10 (SEQ ID NO:20).

[0036] In some examples, the polypeptide of formula (2) comprises in the following amino to carboxyl order:

(a) Monomer 1 comprising:

(i) a first polypeptide comprising an amino acid sequence selected from the group consisting amino acid residues 117-158 of human IL-10 (SEQ ID NO:12); amino acid residues 117-159 of human IL-10 (SEQ ID NO:13); and amino acid residues 117-160 of human IL-10 (SEQ ID NO:14), numbered in accordance with mature human IL-10 (SEQ ID NO:20),

(ii) a first $linker_x$, wherein x = 0 (linker absent) or 1 (linker present);

(iii) a second polypeptide comprising an amino acid sequence selected from the group consisting of amino acid residues 1-116 of human IL-10 (SEQ ID NO:15);

(b) a second $linker_y$, wherein y = 0 (linker absent) or 1 (linker present);

(c) Monomer 2 comprising:

(i) a first polypeptide comprising an amino acid sequence selected from the group consisting amino acid residues 117-158 of human IL-10 (SEQ ID NO:12); amino acid residues 117-159 of human IL-10 (SEQ ID NO:13); and amino acid residues 117-160 of human IL-10 (SEQ ID NO:14), numbered in accordance with mature human IL-10 (SEQ ID NO:20),

(ii) a third $linker_z$, wherein z = 0 (linker absent) or 1 (linker present); and

(iii) a second polypeptide comprising an amino acid sequence selected from the group consisting of amino acid residues 1-116 of human IL-10 (SEQ ID NO:15).

[0037] In some examples, the polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity hIL10 inverted monomer selected from the group consisting of SEQ ID NOS:46-51.

[0038] In some examples, the polypeptide is PEGylated. In some examples, the PEG molecule is linear or branched and has a molecular weight of from about 10kD to about 80kD. In some examples, the PEG molecule is a 40kD branched PEG molecule comprising two 20kD arms. In some examples, the PEG molecule is covalently attached to the N-terminus of the polypeptide.

[0039] In some examples, the polypeptide exhibits cell type biased activity relative to the wild type IL10 species from which the inverted IL10 monomer was derived. In some examples, the polypeptode: (a) exhibits a significant level of at least one anti-inflammatory property of wild type IL10 and (b) exhibits a significantly reduced level of at least one pro-inflammatory property of wild type IL10. In some examples, the at least one anti-inflammatory property is selected from the group consisting of suppression of expression or secretion of IL1b, TNFa or IL6 in a myeloid cell .In some examples, the at least one pro-inflammatory property is selected from the group consisting of suppression of expression or secretion of IFNγ, granzyme A or granzyme B in a T cell. In some examples, the at least one anti-inflammatory property is selected from the group consisting of suppression of expression or secretion of IL1b, TNFa or IL6 in a myeloid cell and the at least one pro-inflammatory property is selected from the group consisting of suppression of expression or secretion of IFNγ, granzyme A or granzyme B in a T cell.

[0040] In some examples, the cell type biased activity is the production of phospho-STAT3. In some examples, the pSTAT3 Emax of the polypeptide is greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, or greater than 70% of the pSTAT3 Emax of wild-type hIL10 in myeloid cells. In some examples, the pSTAT3 Emax in a lymphocyte less than 70%, less than 60%, less than 50%, less than 40%, or less than 30%, of the pSTAT3 Emax of a wild-type hIL10 in the lymphocyte. In some examples, (a) the pSTAT3 Emax of the polypeptide is greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, or greater than 70% of the pSTAT3 Emax of wild-type hIL10 in myeloid cells and (b) the pSTAT3 Emax in a lymphocyte less than 70%, less than 60%, less than 50%, less than 40%, or less than 30%, of the pSTAT3 Emax of a wild-type hIL10 in the lymphocyte.

[0041] In another aspect, provided herein is a nucleic acid molecule having a sequence encoding a polypeptide disclosed herein.

[0041a] Also provided is a nucleic acid sequence encoding a polypeptide as described above or elsewhere herein.

[0042] In another aspect, provided herein is a recombinant vector comprising the nucleic acid molecule disclosed herein operably linked to one or more expression control sequences.

[0042a]Also provided is a recombinant vector comprising the nucleic acid sequence as described above or elsewhere herein operably linked to one or more expression control sequences.

[0043] In another aspect, provided herein is a recombinant cell transformed with the recombinant vector disclosed herein.

[0043a]Also provided is a recombinant cell transformed with the recombinant vector as described above or elsewhere herein.

[0044] In yet another aspect, provided herein is a method of making a polypeptide disclosed herein, the method comprising the steps of (a) culturing the host cell disclosed herein under conditions suitable for the expression of the polypeptide and (b) recovering the polypeptide from the host cell culture, optionally wherein the host cell is a mammalian host cell, or optionally wherein the host cell is a bacterial cell.

[0044a]Also provided is a method of making a polypeptide as described above or elsewhere herein, the method comprising the steps of (a) culturing the host cell as described above or elsewhere herein under conditions suitable for the expression of the polypeptide and (b) recovering the polypeptide from the host cell culture. In some examples, the host cell is a mammalian host cell. In some examples, the host cell is a bacterial cell.

[0045] In yet another aspect, provided herein is a composition comprising a polypeptide disclosed herein, a nucleic acid molecule disclosed herein, or a recombinant vector of claim 11 and one or more pharmaceutically acceptable salts, excipients, and/or diluents.

[0045a] Also provided is a composition comprising a polypeptide as described above or elsewhere herein, a nucleic acid sequence as described above or elsewhere herein, or a recombinant vector as described above or elsewhere herein and one or more pharmaceutically acceptable salts, excipients, and/or diluents.

[0046] In another aspect, provided herein is a use of of the polypeptide disclosed herein or the composition disclosed herein in the manufacture of a medicament for treating a mammalian subject suffering from a disease, disorder or condition, wherein a therapeutically effective amount of the polypeptide or the composition is to be administered to said subject, optionally wherein the disease, disorder or condition is an autoimmune disease, disorder or condition, or optionally wherein the autoimmune disease, disorder or condition is selected from the group consisting of ulcerative colitis, organ rejection, graft versus host disease, autoimmune thyroid disease, multiple sclerosis, allergy, asthma, neurodegenerative diseases including Alzheimer's disease, systemic lupus erythramatosis (SLE), autoinflammatory diseases, inflammatory bowel disease (IBD), Crohn's disease, diabetes including Type 1 or type 2 diabetes, inflammation, autoimmune disease, atopic diseases, paraneoplastic autoimmune diseases, cartilage inflammation, arthritis, rheumatoid arthritis, juvenile arthritis, juvenile rheumatoid arthritis, juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, juvenile ankylosing spondylitis, juvenile enteropathic arthritis, juvenile reactive arthritis, juvenile Reiter's Syndrome, SEA Syndrome (Seronegativity Enthesopathy Arthropathy Syndrome), juvenile dermatomyositis, juvenile psoriatic arthritis, juvenile scleroderma, juvenile systemic lupus erythematosus, juvenile vasculitis, pauciarticular rheumatoidarthritis, polyarticular rheumatoidarthritis, systemic onset rheumatoidarthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, Reiter's syndrome, and SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome),

optionally wherein disease disorder or condition is a cancer associated with chronic inflammation,

optionally wherein the treating prevents the progression of the disease, disorder or condition, or

optionally wherein the treating ameliorates one or more symptoms of the disease, disorder or condition.

[0046a] In another aspect, provided herein is a use of the composition disclosed herein in the manufacture of a medicament for preventing a disease, disorder or condition in a mammalian subject at risk for developing the disease, disorder or condition, wherein a prophylactically effective amount of the composition is to be administered to said subject prior to the onset of symptoms of the disease, disorder or condition,

optionally wherein the disease disorder or condition is a cancer associated with chronic inflammation.

[0046b] In another aspect, provided herein is the polypeptide disclosed herein or the composition disclosed herein, for use in therapy.

[0046c] Also provided is a method of treating a mammalian subject suffering from a disease, disorder or condition, the method comprising administering to said subject a therapeutically effective amount a polypeptide as described above or elsewhere herein, or a composition as described above or elsewhere herein. In some examples, the disease, disorder or condition is an autoimmune disease, disorder or condition. In some examples, the autoimmune disease, disorder or condition is selected from the group consisting of ulcerative colitis, organ rejection, graft versus host disease, autoimmune thyroid disease, multiple sclerosis, allergy, asthma, neurodegenerative diseases including Alzheimer’s disease, systemic lupus erythramatosis (SLE), autoinflammatory diseases, inflammatory bowel disease (IBD), Crohn’s disease, diabetes including Type 1 or type 2 diabetes, inflammation, autoimmune disease, atopic diseases, paraneoplastic autoimmune diseases, cartilage inflammation, arthritis, rheumatoid arthritis, juvenile arthritis, juvenile rheumatoid arthritis, juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, juvenile ankylosing spondylitis, juvenile enteropathic arthritis, juvenile reactive arthritis, juvenile Reiter's Syndrome, SEA Syndrome (Seronegativity Enthesopathy Arthropathy Syndrome), juvenile dermatomyositis, juvenile psoriatic arthritis, juvenile scleroderma, juvenile systemic lupus erythematosus, juvenile vasculitis, pauciarticular rheumatoidarthritis, polyarticular

rheumatoidarthritis, systemic onset rheumatoidarthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, Reiter's syndrome, and SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome). In some examples, disease disorder or condition is a cancer associated with chronic inflammation. In some examples, the treating prevents the progression of the disease, disorder or condition. In some examples, the treating ameliorates one or more symptoms of the disease, disorder or condition.

[0047] Also provided is a method of preventing a disease, disorder or condition in a mammalian subject at risk for developing the disease, disorder or condition, the method comprising administering to said subject a prophylactically effective amount of the composition as described above or elsewhere herein prior to the onset of symptoms of the disease, disorder or condition as described above or elsewhere herein the disease disorder or condition is a cancer associated with chronic inflammation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] FIG. 1 shows thermal stability data relating to an IL10 inverted monomer of the present disclosure as more fully described in Examples. The data indicates that the hIL10 inverted monomer possesses enhanced thermal stability relative to hIL10.

[0049] FIG. 2A shows results of a flow cytometric assay evaluating the level of intracellular STAT3 (y-axis) in human monocytes exposed various concentrations (x-axis) of wild type human IL10 and an inverted hIL10 monomer as more fully described in Examples. FIG. 2B shows results of a flow cytometric assay evaluating the level of intracellular STAT3 (y-axis) in human naïve CD8 T cells exposed various concentrations (x-axis) of wild type human IL10 and an inverted hIL10 monomer as more fully described in Examples. .

[0050] FIG. 3 shows the results of a human monocyte activity assay to detect the secretion of IL-1β (y-axis) in human monocytes exposed to various in concentrations (x-axis) of wild-type hIL10 (h_SM0043_AA) and an inverted monomer of the disclosure (h_DR1061_AA) as more fully described in the Examples.

[0051] Figs. 4A-4C show the secreted levels (y-axes) of IFNγ (FIG. 4A), Granzyme A (FIG. 4B) and Granzyme B (FIG. 4C) in activated human CD8+ T cell in response to various concentrations (x-axes)() of wild-type hIL10 (h_SM0043_AA) and an inverted monomer of the disclosure (h_DR1061_AA) as more fully described in the Examples.

[0187] In some embodiments, the variant inverted monomer comprises the amino acid substitution T100L numbered in accordance with the mature human or mouse IL10 sequence. In some embodiments, the inverted monomer comprises the amino acid sequence:

KSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRNSPGQG
TQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKESLLE
DFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLK**L**LRL
RLRRCHRFLPCEN (SEQ ID NO: 90)

or a sequence having at least 95% sequence identity to SEQ ID NO: 90.

[0188] In some embodiments, one inverted monomer of a dimerized inverted monomer comprises an amino acid substitution(s) either or both of positions D25 or T100 (numbered in accordance with the mature human or mouse IL10 sequence). In some embodiments, each inverted monomer of a dimerized inverted monomer comprises an amino acid substitution one or both of positions D25 or T100 (numbered in accordance with the mature human or mouse IL10 sequence). In some embodiments, one inverted monomer of a dimerized inverted monomer comprises the amino acid substitution D25K (numbered in accordance with the mature human or mouse IL10 sequence). In some embodiments, one inverted monomer of a dimerized inverted monomer comprises the amino acid substitution T100L (numbered in accordance with the mature human or mouse IL10 sequence). In some embodiments, each inverted monomer of a dimerized inverted monomer comprises an amino acid substitution one or both of positions D25 or T100 (numbered in accordance with the mature human or mouse IL10 sequence). In some embodiments, the variant inverted monomer of the comprises the amino acid substitution D25K numbered in accordance with the mature human or mouse IL10 sequence. In some embodiments, the inverted monomer dimer comprises the amino acid sequence:

KSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRNSPGQGT
QSENSCTHFPGNLPNMLR**K**LRDAFSRVKTFFQMKDQLDNLLLKESLLEDF
KGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRL
RRCHRFLPCENKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMT
MKIRNSPGQGTQSENSCTHFPGNLPNMLR**K**LRDAFSRVKTFFQMKDQLD
NLLLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSL
GENLKTLRLRLRRCHRFLPCEN (SEQ ID NO:54), or a sequence having at least 95% sequence identity to SEQ ID NO:54).

100nM for 20 min at 37°C. h_SM0043_AA is a wild type human IL10 sequence expressed in E. coli having the amino acid sequence:

MSPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNL
LLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNS
LGENLKTLRLRLRRCHRFLPCENKSKAVEQVKNAFNKLQEKGIYKAM
SEFDIFINYIEAYMTMKIRN (SEQ ID NO: 166)

The cells were fixed with BD Phosflow™ Fix Buffer I (BD Biosciences, Catalog No. 557870) for 15 mins at 37°C. The cells were then washed and permeabilized with chilled BD Phosflow™ Perm Buffer III (Catalog No. 558050) overnight at -20°C. The cells were washed to remove the permeabilization buffer and blocked with Human Trustain Fcx (Biolegend, Catalog No.422301) and mouse serum for 5 minutes at room temperature. The cells were then treated with the antibody cocktail (listed in Table 14 below) for 1 hour at room temperature.

Table 14. Antibody cocktail.

Fluorochrome	Antibody	Phosphorylation Site	Clone	Catalog#	Vendor
BV480	CD4		L200	566148	BD Biosciences
BV605	CD8a		RPA-T8	301040	Biolegend
BV786	CD56		5.1H11	362550	Biolegend
AF488	*pSTAT3*	Tyr705	D3A7	4323S	CST
PE-Cy5	CD3		UCHT1	555334	BD Biosciences
PerCP-Cy 5.5	CD14		M5E2	301824	Biolegend
APC	CD20		1412	340516	Biolegend

[0327] Following antibody staining, the cells were washed, fixed and ran on the Cytek® Aurora Spectral flow cytometer. The data was analyzed using the FlowJo software (www.flowjo.com). The various cell lineages were gated using their lineage markers and the geometric mean fluorescence intensity of pSTAT3 expression was calculated on FlowJo. The data is shown in Figs. 2A and 2B. The data presented shows that the inverted monomeric IL10 molecule (h_DR1060_AA) exhibits decreased pSTAT3 signalling in monocytes and CD8 T cells as compared to the wild type hIL10 molecule (h_SM0043_AA).

Example 3. Monocyte IL10 Activity Assay:

[0328] Human PBMCs were isolated from LRS chambers obtained from non-smoking human donors by Ficoll® gradient purification. Human monocytes were purified from the PBMCs by positive selection using Human CD14 MicroBeads (Miltenyi Biotech, Catalog No. 130-050-201)

monomeric IL10 molecules (h_DR1060_AA and h_DR1061_AA show decreased pSTAT3 signal in monocytes and CD8 T cells as compared to the native dimeric IL10 molecule (h_DR757_AA).

Example 12. In Vivo Evaluation In DSS Model of Ulcerative Colitis

[0345] The IL10 variants of the present disclosure were evaluated for therapeutic efficacy in a murine DSS model of ulcerative colitis. The murine DSS-induced colitis model is widely used and possess many similarities with human ulcerative colitis. Chassaing, B., et al. (2014) Current Protocols in Immunology 104:15.25.1-15.25.14.

[0346] The canonical sequence of mature (absent the endogenous 18 amino acid signal peptide) wild type murine IL10 molecule (UniProt Reference P18893) is a 160 amino acid polypeptide having the amino acid sequence:

SRGQYSREDNNCTHFPVGQSHMLLELRTAFSQVKTFFQTKDQLDNILLTDSLMQDFKGYL
GCQALSEMIQFYLVEVMPQAEKHGPEIKEHLNSLGEKLKTLRMRLRRCHRFLPCENKSKA
VEQVKSDFNKLQDQGVYKAMNEFDIFINCIEAYMMIKMK (SEQ ID NO: 165)

When referencing amino acid substitutions or deletions in the murine IL10 variant molecules described herein are made in reference SEQ ID NO: 165.

[0347] The following Table 17 describes the pegylated murine IL10 (mIL10) test articles were used in the DSS study.

Table 17. DSS Study Murine Test Articles

Name	Parent Polypeptide (SEQ ID)	PEGylation Location and Description
m_DR756_AA_PEG	m_DR756_AA	N-terminus, 20kDa Linear PEG
m_WC161_AA_PEG	m_WC161_AA	N-terminus, 40 kDa (branched 2x20) PEG

[0348] The amino acid sequences of the parent polypeptides of the m_DR756_AA_PEG and m_WC161_AA_PEG test articles are provided in Table 18 below:

Table 18. Parent Polypeptides of PEGylated DSS Study Articles

SEQ ID NO	Name	Description	Amino Acid Sequence
163	m_DR756_AA	Alanine;8xHis (SEQ ID NO: 140) chelating peptide; Gly-Ser linker; ΔS1/ΔR2/ΔG3 mIL10	AHHHHHHHHGSQYSREDNNCTHFPVGQ SHMLLELRTAFSQVKTFFQTKDQLDNIL LTDSLMQDFKGYLGCQALSEMIQFYLVE VMPQAEKHGPEIKEHLNSLGEKLKTLRM RLRRCHRFLPCENKSKAVEQVKSDFNKL QDQGVYKAMNEFDIFINCIEAYMMIKM KS
164	m_WC161_AA	AAs 116-160 wt mIL10; AAs 1-115 wt mIL10;	NKSKAVEQVKSDFNKLQDQGVYKAMN EFDIFINAIEAYMMIKMKSSRGQYSREDN

		GGS linker; 8xHis (SEQ ID NO: 140) chelating peptide	NCTHFPVGQSHMLLELRTAFSQVKTFFQ TKDQLDNILLTDSLMQDFKGYLGCQALS EMIQFYLVEVMPQAEKHGPEIKEHLNSL GEKLKTLRMRLRRCHRFLPCENGGSHH HHHHHH

[0349] Briefly, the DSS study was conducted as follows. Female C57Bl/6 mice (Jackson Laboratories) were administered 2.5% DSS, ad libitum, in drinking water on study days 0-6. Mice given normal water served as a negative disease control. Mice were treated with either PBS, m_DR756_AA_PEG (3 or 10 micrograms administered subcutaneously every other day) or m_WC161_AA_PEG (10 or 30 micrograms administered subcutaneously every other day) in accordance with the following Table 19.

Table 19. DSS Study Treatment Groups

Group	DSS	Treatment/Test Article	Dose [ug]	Schedule (Study Days)
A	Water	n/a	-	-
B	2.5%	PBS	-	D0, D2, D4, D6, D8
C	2.5%	m_DR756_AA_PEG	3	D0, D2, D4, D6, D8
D	2.5%	m_DR756_AA_PEG	10	D0, D2, D4, D6, D8
E	2.5%	m_WC161_AA_PEG	10	D0, D2, D4, D6, D8
F	2.5%	m_WC161_AA_PEG	30	D0, D2, D4, D6, D8

[0350] The bodyweights of the mice were evaluated throughout the study. Mice were weighed three days prior to initiation of DSS (Study Day -3) and after DSS administration and test article treatment. The data relating to bodyweight for each treatment group is provided in Figures 13 of the attached drawings. Both average body weight and average % body weight +/- SEM are shown. Percent body weight was calculated as = (Body weight on Study Day X – Baseline body weight on Study Day -3) / (Baseline body weight on Study Day -3). Terminal bodyweight of the mice was evaluated on Study Day 15 and the average % body weight +/- SEM on day 15 is shown in Figure 14 of the attached drawings. Significance was determined by One-Way ANOVA with Holm-Šídák's multiple comparisons test. ****$p<0.0001$

[0351] On Study Day 15, terminal blood was harvested via cardiac puncture and collected into serum collection tubes. In-life and terminal serum was isolated by centrifuging clotted blood for 5 minutes at 10,000 RPM. Serum was collected after centrifugation and placed on ice temporarily before freezing for later analysis. Serum cytokines were assessed using Meso Scale Discovery multiplex kits (Meso Scale Discovery) in substantial accordance with the manufacturer's instructions.