

UPAR ANTIBODIES AND FUSION PROTEINS WITH THE SAME**RELATED APPLICATION**

This application claims the benefit of U.S. Provisional Patent Application Serial No. 63/165,313 filed March 24, 2021, the entire disclosure of which is hereby incorporated herein
5 by reference.

BACKGROUND

Immunotherapy is a rapidly emerging treatment modality for cancer. Immunotherapies fall into the general categories of: (i) immune checkpoint molecule modulators capable of agonizing or antagonizing regulatory molecules as appropriate to
10 enhance a desirable immune response (e.g. agonizing stimulatory immune receptors) or to inhibit an undesirable immune response (e.g. antagonizing inhibitory immune checkpoint molecules); (ii) cytokines, protein molecules that help regulate and direct the immune system; and (iii) cancer vaccines, molecules (or combinations) that generate immunological memory responses to specific antigens/pathogens. While promising immunotherapies are now in the
15 clinic with an encouraging pipeline of new drugs/agents under development, it would be desirable to develop new immunotherapies for use on their own or in combination with other categories of immunotherapy, targeted therapies and conventional cytotoxic therapies.

SUMMARY

In one aspect, the disclosure provides an isolated antigen binding protein that binds
20 specifically to urokinase plasminogen activator receptor (uPAR), comprising:

(a) an antibody heavy chain variable (VH) domain comprising:

- i) an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), GFSLTNYG (SEQ ID NO: 11), GNTFTDYG (SEQ ID NO: 19), GTTFTDYG (SEQ ID NO: 41), or GYTFTSYG (SEQ ID NO: 27);
- 25 ii) an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), IWSDGGT (SEQ ID NO: 12), INTNTGEP (SEQ ID NO: 20), or IYPRSGNT (SEQ ID NO: 28); and
- iii) an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5), ARGGRSDLFAY (SEQ ID NO: 13), AHYSFDY (SEQ ID NO: 21), or AGKDYGSTYADY (SEQ ID NO: 29); and

(b) an antibody light chain variable (VL) domain comprising:

iv) an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), QSIVHSNGNTY (SEQ ID NO: 14), ENIYSN (SEQ ID NO: 22), SSVSSRY (SEQ ID NO: 30);

v) an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), KVS (SEQ ID NO: 15),
5 AAT (SEQ ID NO: 23), or GTS (SEQ ID NO: 31); and

vi) an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8), FQGSHVPYT (SEQ ID NO: 16), QHFWGTPWT (SEQ ID NO: 24), QQYHSDPLT (SEQ ID NO: 32).

In certain embodiments, the VH domain comprises an amino acid sequence of SEQ ID
10 NO: 33, SEQ ID NO: 34, SEQ ID NO: 37, SEQ ID NO: 42, or SEQ ID NO: 43, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, or SEQ ID NO: 44.

In certain embodiments of the antigen binding protein:

a) the VH domain comprises an HCDR1 amino acid sequence of GFNIKDEY (SEQ
15 ID NO: 3), an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5); and

b) the VL domain comprises an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), and an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8).

20 In certain embodiments of the antigen binding protein:

a) the VH domain comprises an HCDR1 amino acid sequence of GFSLTNYG (SEQ ID NO: 11), an HCDR2 amino acid sequence of IWSDGGT (SEQ ID NO: 12), an HCDR3 amino acid sequence of ARGGRSDFAY (SEQ ID NO: 13); and

b) the VL domain comprises an LCDR1 amino acid sequence of QSIVHSNGNTY
25 (SEQ ID NO: 14), an LCDR2 amino acid sequence of KVS (SEQ ID NO: 15), and an LCDR3 amino acid sequence of FQGSHVPYT (SEQ ID NO: 16).

In certain embodiments of the antigen binding protein:

a) the VH domain comprises an HCDR1 amino acid sequence of GNTFTDYG (SEQ ID NO: 19), or GTTFTDYG (SEQ ID NO: 41), an HCDR2 amino acid sequence of
30 INTNTGEP (SEQ ID NO: 20), an HCDR3 amino acid sequence of AHYSFDY (SEQ ID NO: 21); and

b) the VL domain comprises an LCDR1 amino acid sequence of ENIYSN (SEQ ID NO: 22), an LCDR2 amino acid sequence of AAT (SEQ ID NO: 23), and an LCDR3 amino acid sequence of QHFWGTPWT (SEQ ID NO: 24).

In certain embodiments of the antigen binding protein:

5 a) the VH domain comprises an HCDR1 amino acid sequence of GYTFTSYG (SEQ ID NO: 27), an HCDR2 amino acid sequence of IYPRSGNT (SEQ ID NO: 28), an HCDR3 amino acid sequence of AGKDYGSTYADY (SEQ ID NO: 29); and

b) the VL domain comprises an LCDR1 amino acid sequence of SSVSSRY (SEQ ID NO: 30), an LCDR2 amino acid sequence of GTS (SEQ ID NO: 31), and an LCDR3 amino acid sequence of QQYHSDPLT (SEQ ID NO: 32).

In certain embodiments of the antigen binding protein:

a) the VH domain comprises an amino acid sequence of SEQ ID NO: 33, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35;

15 b) the VH domain comprises an amino acid sequence of SEQ ID NO: 33, and the VL domain comprises an amino acid sequence of SEQ ID NO: 36;

c) the VH domain comprises an amino acid sequence of SEQ ID NO: 34, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35;

d) the VH domain comprises an amino acid sequence of SEQ ID NO: 34, and the VL domain comprises an amino acid sequence of SEQ ID NO: 36;

20 e) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 38;

f) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 39;

25 g) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 40;

h) the VH domain comprises an amino acid sequence of SEQ ID NO: 42, and the VL domain comprises an amino acid sequence of SEQ ID NO: 44; or

i) the VH domain comprises an amino acid sequence of SEQ ID NO: 43, and the VL domain comprises an amino acid sequence of SEQ ID NO: 44.

30 In certain embodiments, the VH domain and VL domain are attached with an amino acid linker.

In certain embodiments, the amino acid linker comprises (GGGS)_n (SEQ ID NO: 148), wherein n is an integer between 1 and 5.

In certain embodiments, the amino acid linker comprises the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO: 149),
5 GGSGGGGSGGGSGGGGSGGGSGGGSGG (SEQ ID NO: 150), or GGGSGGGGSG (SEQ ID NO: 246).

In certain embodiments, the antigen binding protein comprises a Fab fragment, a F(ab')₂ fragment, an Fd fragment, an Fv fragment, a single chain Fv (scFv), a dAb fragment, a single domain antibody, or a nanobody.

10 In certain embodiments, the antigen binding protein comprises the amino acid sequence of any one of SEQ ID NOs: 56-71.

In certain embodiments, the antigen binding protein further comprises the amino acid sequence of any one of SEQ ID NOs: 72-78.

In certain embodiments, the antigen binding protein comprises an antibody heavy
15 chain (HC) amino acid sequence of SEQ ID NO: 45, 46, 49, 53, or 54, and an antibody light chain (LC) amino acid sequence of SEQ ID NO: 47, 48, 50, 51, 52, and 55.

In certain embodiments of the antigen binding protein:

- a) the antibody HC comprises the amino acid sequence of SEQ ID NO: 45, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 47;
- 20 b) the antibody HC comprises the amino acid sequence of SEQ ID NO: 45, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 48;
- c) the antibody HC comprises the amino acid sequence of SEQ ID NO: 46, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 47;
- d) the antibody HC comprises the amino acid sequence of SEQ ID NO: 46, and the
25 antibody LC comprises the amino acid sequence of SEQ ID NO: 48;
- e) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 50;
- f) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 51;
- 30 g) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 52;

h) the antibody HC comprises the amino acid sequence of SEQ ID NO: 53, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 55; or

i) the antibody HC comprises the amino acid sequence of SEQ ID NO: 54, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 55.

5 In certain embodiments, the antigen binding protein comprises an amino acid sequence of SEQ ID NO: 189.

In certain embodiments, the antigen binding protein comprises an amino acid sequence of SEQ ID NO: 213.

10 In certain embodiments, the antigen binding protein comprises: a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 178; and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159.

In certain embodiments, the antigen binding protein comprises: a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 193; and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 161.

15 In certain embodiments, the antigen binding protein comprises: a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 179; and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159.

20 In certain embodiments, the antigen binding protein comprises: a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 194; and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 162.

In certain embodiments, the antigen binding protein comprises: a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 238; a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159; and a third polypeptide chain comprising an amino acid sequence of SEQ ID NO: 239.

25 In certain embodiments, the antigen binding protein binds human uPAR with a KD of less than about 5×10^{-8} M, less than about 1×10^{-8} M, less than about 5×10^{-9} M, less than about 1×10^{-9} M, less than about 5×10^{-10} M, or less than about 1×10^{-10} M.

In certain embodiments, the antigen binding protein binds human uPAR in the presence of a uPAR ligand.

30 In certain embodiments, the antigen binding protein thereof binds uPAR ligand-bound human uPAR.

In certain embodiments, the antigen binding protein binds human uPAR on human uPAR-expressing cells, thereby producing a fluorescent signal detected with a fluorescently-labelled antibody that binds to the antigen binding protein, with an EC50 value of about 0.1 nM to about 3.0 nM.

5 In certain embodiments, the antigen binding protein binds human uPAR on human uPAR-expressing cells in the presence of a uPAR ligand.

In certain embodiments, the antigen binding protein binds a human uPAR DII-DIII domain or a human uPAR DIII domain.

10 In one aspect, the disclosure provides an isolated antigen binding protein thereof that competes for binding with the antigen binding protein recited above.

In another aspect, the disclosure provides an isolated antigen binding protein thereof that binds the same epitope as the antigen binding protein recited above.

In one aspect, the disclosure provides a pharmaceutical composition comprising the antigen binding protein recited above, and a pharmaceutically acceptable carrier or diluent.

15 In one aspect, the disclosure provides an isolated nucleic acid molecule comprising a polynucleotide sequence that encodes the antigen binding protein recited above.

In one aspect, the disclosure provides an expression vector comprising the polynucleotide sequence recited above.

20 In one aspect, the disclosure provides a host cell comprising the expression vector recited above.

In another aspect, the disclosure provides a method of producing the antigen binding protein described above, comprising culturing the host cell recited above under conditions to express the antigen binding protein.

25 In certain embodiment, the method further comprises isolating the antigen binding protein from the host cell.

In one aspect, the disclosure provides a use of an antigen binding protein that binds specifically to uPAR, for treating or preventing a disease or disorder associated with uPAR activity or expression, the use comprising administering a therapeutically effective amount of the pharmaceutical composition recited above to a subject in need of such treatment.

In one aspect, the disclosure provides a method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition recited above.

In one aspect, the disclosure provides a fusion protein comprising: (i) the antigen
5 binding protein recited above; and (ii) a cytokine or variant thereof.

In certain embodiments of the fusion protein, the antigen binding protein and the cytokine or variant thereof are linked with an amino acid linker.

In certain embodiments of the fusion protein, the cytokine or variant thereof is selected from the group consisting of IL-2, circularly permuted IL-2 (cpIL-2), IL-15,
10 circularly permuted IL15 (cpIL-15), IL-6, circularly permuted IL-6 (cpIL-6), IL-10, or IL-12.

In certain embodiments of the fusion protein, the cytokine or variant thereof comprises at least one interleukin-12 (IL-12) subunit.

In certain embodiments of the fusion protein, the IL-12 subunit comprises one or both of p35 and p40.

15 In certain embodiments of the fusion protein, the p35 comprises one or more amino acid substitutions at positions M12, S27, N28, Q35, F39, S44, C74, M111, V114, M119, M145, F150, L161, and D188 of SEQ ID NO: 81.

In certain embodiments of the fusion protein, the amino acid substitution comprises M12S; S27D; N28R; Q35E; F39D or F39H; S44K; C74K, C74D, C74W, C74Y, or C74Q;
20 M111F or M111W; V114I; M119L; M145L; F150Y; L161D or L161Q; D188N; or a combination thereof.

In certain embodiments of the fusion protein, the amino acid substitution comprises N28R and Q35E.

In certain embodiments of the fusion protein, the amino acid substitution comprises
25 S27D and D188N.

In certain embodiments of the fusion protein, the amino acid substitution comprises C74K.

In certain embodiments of the fusion protein, the amino acid substitution comprises S27D, C74K, and D188N.

30 In certain embodiments of the fusion protein, the p35 comprises an amino acid sequence of SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.

In certain embodiments of the fusion protein, the p40 comprises one or more amino acid substitution at positions C177 and C252 of SEQ ID NO: 84.

In certain embodiments of the fusion protein, the C177 amino acid substitution is selected from the group consisting of C177S, C177F, C177M, C177H, C177I, or C177Q.

5 In certain embodiments of the fusion protein, the C252 amino acid substitution is C252S.

In certain embodiments of the fusion protein, the p40 comprises an amino acid sequence of SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87.

10 In certain embodiments of the fusion protein, the cytokine or variant thereof comprises a single-chain IL-12 (scIL-12) comprising a p35 and a p40 subunit attached by an amino acid linker.

In certain embodiments of the fusion protein, the amino acid linker comprises the sequence GGS GGGGSGG (SEQ ID NO: 156).

15 In certain embodiments of the fusion protein, the scIL-12 comprises an amino acid sequence of SEQ ID NO: 88 or SEQ ID NO: 89.

In one aspect, the disclosure provides a pharmaceutical composition comprising the fusion protein recited above, and a pharmaceutically acceptable carrier or diluent.

In one aspect, the disclosure provides an isolated nucleic acid molecule comprising a polynucleotide sequence that encodes the fusion protein recited above.

20 In one aspect, the disclosure provides an expression vector comprising the polynucleotide sequence recited above.

In one aspect, the disclosure provides a host cell comprising the expression vector recited above.

25 In another aspect, the disclosure provides a method of producing the fusion protein recited above, comprising culturing the host cell recited above under conditions to express the fusion protein.

In certain embodiments, the method further comprises isolating the fusion protein from the host cell.

30 In one aspect, the disclosure provides a use of fusion protein that binds specifically to uPAR, for treating or preventing a disease or disorder associated with uPAR activity or expression, the use comprising administering a therapeutically effective amount of the

pharmaceutical composition recited above or the fusion protein recited above to a subject in need of such treatment.

In one aspect, the disclosure provides a method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition recited above or the fusion protein recited above.

In certain embodiments, the pharmaceutical composition or fusion protein is administered simultaneously with a cytokine or variant thereof or a second fusion protein, wherein the second fusion protein comprises a different cytokine or variant thereof from the first fusion protein.

In certain embodiments, the pharmaceutical composition or fusion protein is administered sequentially a cytokine or variant thereof or a second fusion protein, wherein the second fusion protein comprises a different cytokine or variant thereof from the first fusion protein.

In certain embodiments, the cytokine or variant thereof comprises a IL-12 p35 subunit or an IL-12 p40 subunit.

In certain embodiments, the fusion protein clearance rate in a tumor of the patient is slower than the fusion protein clearance rate in serum of the patient.

In one aspect, the disclosure provides a method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a fusion protein and a non-fusion IL-12 subunit, wherein:

(a) the fusion protein comprises:

(i) an antigen binding protein that binds specifically to urokinase plasminogen activator receptor (uPAR); and

(ii) a IL-12 p35 subunit or variant thereof, or a IL-12 p40 subunit or variant thereof; and

(b) the non-fusion IL-12 subunit comprising a IL-12 p35 subunit or variant thereof, or a IL-12 p40 subunit or variant thereof,

wherein when the fusion protein comprises a IL-12 p35 subunit or variant thereof, the non-fusion IL-12 subunit comprises a IL-12 p40 subunit or variant thereof, and when the fusion protein comprises a IL-12 p40 subunit or variant thereof, the non-fusion IL-12 subunit comprises a IL-12 p35 subunit or variant thereof.

In certain embodiments, the fusion protein and non-fusion IL-12 subunit are administered sequentially.

In certain embodiments, one or both of the fusion protein and non-fusion IL-12 subunit are administered intravenously.

5 In certain embodiments, the fusion protein is administered first, followed by administering the non-fusion IL-12 subunit after a sufficient amount of time has elapsed for the fusion protein to accumulate in a tumor of the patient.

In certain embodiments, the fusion protein is administered first, followed by administering the non-fusion IL-12 subunit after a sufficient amount of time has elapsed for
10 the fusion protein concentration to substantially reduce in serum of the patient.

In one aspect, the disclosure provides a method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a first fusion protein and a second fusion protein, wherein:

(a) the first fusion protein comprises:

15 (i) an antigen binding protein that binds specifically to a first epitope on urokinase plasminogen activator receptor (uPAR); and

(ii) an IL-12 p35 subunit or variant thereof; and

(b) the second fusion protein comprises:

(i) an antigen binding protein that binds specifically to a second epitope on uPAR; and

20 (ii) an IL-12 p40 subunit or variant thereof.

In certain embodiments, the first epitope and second epitope are the same.

In certain embodiments, the first epitope and second epitope are different.

In certain embodiments, the first fusion protein and second fusion protein are administered sequentially.

25 In certain embodiments, one or both of the first fusion protein and second fusion protein are administered intravenously.

In certain embodiments, the first fusion protein is administered first, followed by administering the second fusion protein after a sufficient amount of time has elapsed for the first fusion protein to accumulate in a tumor of the patient.

In certain embodiments, the first fusion protein is administered first, followed by administering the second fusion protein after a sufficient amount of time has elapsed for the first fusion protein concentration to substantially reduce in serum of the patient.

5 In certain embodiments, the second fusion protein is administered first, followed by administering the first fusion protein after a sufficient amount of time has elapsed for the second fusion protein to accumulate in a tumor of the patient.

In certain embodiments, the second fusion protein is administered first, followed by administering the first fusion protein after a sufficient amount of time has elapsed for the second fusion protein concentration to substantially reduce in serum of the patient.

10 In certain embodiments, one or both of the first fusion protein and second fusion protein comprise an antigen binding protein comprising:

a) a VH domain comprising an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5); and

15 b) a VL domain comprising an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), and an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8).

In certain embodiments, one or both of the first fusion protein and second fusion protein comprise an antigen binding protein comprising:

20 a) a VH domain comprising an HCDR1 amino acid sequence of GNTFTDYG (SEQ ID NO: 19), or GTTFTDYG (SEQ ID NO: 41), an HCDR2 amino acid sequence of INTNTGEP (SEQ ID NO: 20), an HCDR3 amino acid sequence of AHYSFDY (SEQ ID NO: 21); and

25 b) a VL domain comprising an LCDR1 amino acid sequence of ENIYSN (SEQ ID NO: 22), an LCDR2 amino acid sequence of AAT (SEQ ID NO: 23), and an LCDR3 amino acid sequence of QHFWGTPWT (SEQ ID NO: 24).

In certain embodiments, one or both of the first fusion protein and second fusion protein comprise an antigen binding protein comprising:

30 a) a VH domain comprising an HCDR1 amino acid sequence of GYTFTSYG (SEQ ID NO: 27), an HCDR2 amino acid sequence of IYPRSGNT (SEQ ID NO: 28), an HCDR3 amino acid sequence of AGKDYGSTYADY (SEQ ID NO: 29); and

b) a VL domain comprising an LCDR1 amino acid sequence of SSVSSRY (SEQ ID NO: 30), an LCDR2 amino acid sequence of GTS (SEQ ID NO: 31), and an LCDR3 amino acid sequence of QQYHSDPLT (SEQ ID NO: 32).

5 In one aspect, the disclosure provides a combination comprising a first fusion protein and a second fusion protein, wherein:

(a) the first fusion protein comprises:

(i) an antigen binding protein that binds specifically to a first epitope on urokinase plasminogen activator receptor (uPAR); and

(ii) a IL-12 p35 subunit or variant thereof; and

10 (b) the second fusion protein comprises:

(i) an antigen binding protein that binds specifically to a second epitope on uPAR; and

(ii) a IL-12 p40 subunit or variant thereof.

In certain embodiments, the first epitope and second epitope are the same.

In certain embodiments, the first epitope and second epitope are different.

15 In one aspect, the disclosure provides a fusion protein comprising: (i) an antigen binding protein that binds specifically to urokinase plasminogen activator receptor (uPAR); and (ii) a cytokine or variant thereof.

In certain embodiments, the antigen binding protein and the cytokine or variant thereof are linked with an amino acid linker.

20 In certain embodiments, the cytokine or variant thereof is selected from the group consisting of IL-2, circularly permuted IL-2 (cpIL-2), IL-15, circularly permuted IL15 (cpIL-15), IL-6, circularly permuted IL-6 (cpIL-6), IL-10, or IL-12.

In certain embodiments, the cytokine or variant thereof comprises at least one interleukin-12 (IL-12) subunit.

25 In certain embodiments, the IL-12 subunit comprises one or both of p35 and p40.

In certain embodiments, the p35 comprises one or more amino acid substitutions at positions M12, S27, N28, Q35, F39, S44, C74, M111, V114, M119, M145, F150, L161, and D188 of SEQ ID NO: 81.

30 In certain embodiments, the amino acid substitution comprises M12S; S27D; N28R; Q35E; F39D or F39H; S44K; C74K, C74D, C74W, C74Y, or C74Q; M111F or M111W; V114I; M119L; M145L; F150Y; L161D or L161Q; D188N; or a combination thereof.

In certain embodiments, the amino acid substitution comprises N28R and Q35E.

In certain embodiments, the amino acid substitution comprises S27D and D188N.

In certain embodiments, the amino acid substitution comprises C74K.

5 In certain embodiments, the amino acid substitution comprises S27D, C74K, and D188N.

In certain embodiments, the p35 comprises an amino acid sequence of SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.

In certain embodiments, the p40 comprises one or more amino acid substitution at positions C177 and C252 of SEQ ID NO: 84.

10 In certain embodiments, the C177 amino acid substitution is selected from the group consisting of C177S, C177F, C177M, C177H, C177I, or C177Q.

In certain embodiments, the C252 amino acid substitution is C252S.

In certain embodiments, the p40 comprises an amino acid sequence of SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87.

15 In certain embodiments, the cytokine or variant thereof comprises a single-chain IL-12 (scIL-12) comprising a p35 and a p40 subunit attached by an amino acid linker.

In certain embodiments, the amino acid linker comprises the sequence GGSGGGGSGG (SEQ ID NO: 156).

20 In certain embodiments, the scIL-12 comprises an amino acid sequence of SEQ ID NO: 88 or SEQ ID NO: 89.

In certain embodiments, the antigen binding protein comprises:

(a) an antibody heavy chain variable (VH) domain comprising:

25 i) an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), GFSLTNYG (SEQ ID NO: 11), GNTFTDYG (SEQ ID NO: 19), GTTFTDYG (SEQ ID NO: 41), or GYTFTSYG (SEQ ID NO: 27);

ii) an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), IWSDGGT (SEQ ID NO: 12), INTNTGEP (SEQ ID NO: 20), or IYPRSGNT (SEQ ID NO: 28); and

30 iii) an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5), ARGGRSDFAY (SEQ ID NO: 13), AHYSFDY (SEQ ID NO: 21), or AGKDYGSTYADY (SEQ ID NO: 29); and

(b) an antibody light chain variable (VL) domain comprising:

iv) an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), QSIVHSNGNTY (SEQ ID NO: 14), ENIYSN (SEQ ID NO: 22), SSVSSRY (SEQ ID NO: 30);

v) an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), KVS (SEQ ID NO: 15), AAT (SEQ ID NO: 23), or GTS (SEQ ID NO: 31); and

5 vi) an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8), FQGSHVPYT (SEQ ID NO: 16), QHFWGTPWT (SEQ ID NO: 24), QQYHSDPLT (SEQ ID NO: 32).

In certain embodiments, the VH domain comprises an amino acid sequence of SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 37, SEQ ID NO: 42, or SEQ ID NO: 43, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, or SEQ ID NO: 44.

In certain embodiments of the fusion protein:

a) the VH domain comprises an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5); and

b) the VL domain comprises an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), and an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8).

In certain embodiments of the fusion protein:

20 a) the VH domain comprises an HCDR1 amino acid sequence of GFSLTNYG (SEQ ID NO: 11), an HCDR2 amino acid sequence of IWSDDGGT (SEQ ID NO: 12), an HCDR3 amino acid sequence of ARGGRSDFAY (SEQ ID NO: 13); and

b) the VL domain comprises an LCDR1 amino acid sequence of QSIVHSNGNTY (SEQ ID NO: 14), an LCDR2 amino acid sequence of KVS (SEQ ID NO: 15), and an LCDR3 amino acid sequence of FQGSHVPYT (SEQ ID NO: 16).

In certain embodiments of the fusion protein:

a) the VH domain comprises an HCDR1 amino acid sequence of GNTFTDYG (SEQ ID NO: 19), or GTTFTDYG (SEQ ID NO: 41), an HCDR2 amino acid sequence of INTNTGEP (SEQ ID NO: 20), an HCDR3 amino acid sequence of AHYSFDY (SEQ ID NO: 21); and

b) the VL domain comprises an LCDR1 amino acid sequence of ENIYSN (SEQ ID NO: 22), an LCDR2 amino acid sequence of AAT (SEQ ID NO: 23), and an LCDR3 amino acid sequence of QHFWGTPWT (SEQ ID NO: 24).

In certain embodiments of the fusion protein:

5 a) the VH domain comprises an HCDR1 amino acid sequence of GYTFTSYG (SEQ ID NO: 27), an HCDR2 amino acid sequence of IYPRSGNT (SEQ ID NO: 28), an HCDR3 amino acid sequence of AGKDYGSTYADY (SEQ ID NO: 29); and

b) the VL domain comprises an LCDR1 amino acid sequence of SSVSSRY (SEQ ID NO: 30), an LCDR2 amino acid sequence of GTS (SEQ ID NO: 31), and an LCDR3 amino acid sequence of QQYHSDPLT (SEQ ID NO: 32).

In certain embodiments of the fusion protein:

a) the VH domain comprises an amino acid sequence of SEQ ID NO: 33, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35;

15 b) the VH domain comprises an amino acid sequence of SEQ ID NO: 33, and the VL domain comprises an amino acid sequence of SEQ ID NO: 36;

c) the VH domain comprises an amino acid sequence of SEQ ID NO: 34, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35;

d) the VH domain comprises an amino acid sequence of SEQ ID NO: 34, and the VL domain comprises an amino acid sequence of SEQ ID NO: 36;

20 e) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 38;

f) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 39;

25 g) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 40;

h) the VH domain comprises an amino acid sequence of SEQ ID NO: 42, and the VL domain comprises an amino acid sequence of SEQ ID NO: 44; or

i) the VH domain comprises an amino acid sequence of SEQ ID NO: 43, and the VL domain comprises an amino acid sequence of SEQ ID NO: 44.

30 In certain embodiments, the VH domain and VL domain are attached with an amino acid linker.

In certain embodiments, the amino acid linker comprises (GGGGS)_n (SEQ ID NO: 148), wherein n is an integer between 1 and 5.

In certain embodiments, the amino acid linker comprises the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO: 149),
5 GGSGGGGSGGGSGGGGSGGGGSGGGSGG (SEQ ID NO: 150), or GGGSGGGGSG (SEQ ID NO: 246).

In certain embodiments, the antigen binding protein comprises a Fab fragment, a F(ab')₂ fragment, an Fd fragment, an Fv fragment, a single chain Fv (scFv), a dAb fragment, a single domain antibody, or a nanobody.

10 In certain embodiments, the antigen binding protein comprises the amino acid sequence of any one of SEQ ID NOs: 56-71.

In certain embodiments, the antigen binding protein further comprises the amino acid sequence of any one of SEQ ID NOs: 72-78.

In certain embodiments, the fusion protein comprises an antibody heavy chain (HC) amino acid sequence of SEQ ID NO: 45, 46, 49, 53, or 54, and an antibody light chain (LC) amino acid sequence of SEQ ID NO: 47, 48, 50, 51, 52, and 55.

In certain embodiments of the fusion protein:

- a) the antibody HC comprises the amino acid sequence of SEQ ID NO: 45, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 47;
- 20 b) the antibody HC comprises the amino acid sequence of SEQ ID NO: 45, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 48;
- c) the antibody HC comprises the amino acid sequence of SEQ ID NO: 46, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 47;
- d) the antibody HC comprises the amino acid sequence of SEQ ID NO: 46, and the
25 antibody LC comprises the amino acid sequence of SEQ ID NO: 48;
- e) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 50;
- f) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 51;
- 30 g) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 52;

h) the antibody HC comprises the amino acid sequence of SEQ ID NO: 53, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 55; or

i) the antibody HC comprises the amino acid sequence of SEQ ID NO: 54, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 55.

5 In certain embodiments, the fusion protein comprises an amino acid sequence of SEQ ID NO: 189.

 In certain embodiments, the fusion protein comprises an amino acid sequence of SEQ ID NO: 213.

10 In certain embodiments, the fusion protein comprises: a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 178; and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159.

 In certain embodiments, the fusion protein comprises: a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 193; and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 161.

15 In certain embodiments, the fusion protein comprises: a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 179; and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159.

 In certain embodiments, the fusion protein comprises: a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 194; and a second polypeptide chain
20 comprising an amino acid sequence of SEQ ID NO: 162.

 In certain embodiments, the fusion protein comprises: a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 238; a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159; and a third polypeptide chain comprising an amino acid sequence of SEQ ID NO: 239.

25 In certain embodiments, the antigen binding protein binds human uPAR with a KD of less than about 5×10^{-8} M, less than about 1×10^{-8} M, less than about 5×10^{-9} M, less than about 1×10^{-9} M, less than about 5×10^{-10} M, or less than about 1×10^{-10} M.

 In certain embodiments, the antigen binding protein binds human uPAR in the presence of a uPAR ligand.

30 In certain embodiments, the antigen binding protein binds uPAR ligand-bound human uPAR.

In certain embodiments, the antigen binding protein binds human uPAR on human uPAR-expressing cells, thereby producing a fluorescent signal detected with a fluorescently-labelled antibody that binds to the antibody or fragment thereof, with an EC50 value of about 0.1 nM to about 3.0 nM.

5 In certain embodiments, the antigen binding protein binds human uPAR on human uPAR-expressing cells in the presence of a uPAR ligand.

In certain embodiments, the antigen binding protein binds a human uPAR DII-DIII domain or a human uPAR DIII domain.

In one aspect, the disclosure provides an engineered IL-12 p35 polypeptide,
10 comprising one or more amino acid substitutions at positions S27, N28, Q35, F39, S44, C74, M111, V114, M119, M145, F150, and L161 of SEQ ID NO: 81.

In certain embodiments, the engineered IL-12 p35 polypeptide further comprises one or both of a S27 and D188 amino acid substitution.

In certain embodiments, the amino acid substitution comprises M12S; S27D; N28R;
15 Q35E; F39D or F39H; S44K; C74K, C74D, C74W, C74Y, or C74Q; M111F or M111W; V114I; M119L; M145L; F150Y; L161D or L161Q; D188N; or a combination thereof.

In certain embodiments, the amino acid substitution comprises N28R and Q35E.

In certain embodiments, the amino acid substitution comprises C74K.

In certain embodiments, the amino acid substitution comprises S27D, C74K, and
20 D188N.

In certain embodiments, the engineered IL-12 p35 polypeptide comprises an amino acid sequence of SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.

In another aspect, the disclosure provides a fusion protein comprising: (i) the engineered IL-12 p35 polypeptide recited above; and (ii) one or more fusion partners.

25 In certain embodiments, the one or more fusion partners comprise a moiety that reduces aggregation of the engineered IL-12 p35 polypeptide.

In certain embodiments, the one or more fusion partners comprise a moiety that increases expression of the engineered IL-12 p35 polypeptide.

In certain embodiments, the one or more fusion partners comprise a moiety that
30 increases serum half-life of the engineered IL-12 p35 polypeptide.

In certain embodiments, the one or more fusion partners comprise a targeting moiety.

In certain embodiments, the one or more fusion partners comprise a serum albumin, polyethylene glycol (PEG), or an antigen binding protein.

In certain embodiments, the antigen binding protein binds specifically to urokinase plasminogen activator receptor (uPAR).

5 In one aspect, the disclosure provides an engineered IL-12 p40 polypeptide, comprising one or more amino acid substitutions at positions C177 and C252 of SEQ ID NO: 84.

In certain embodiments, the C177 amino acid substitution is selected from the group consisting of C177S, C177F, C177M, C177H, C177I, or C177Q.

10 In certain embodiments, the C252 amino acid substitution is C252S.

In certain embodiments, the p40 comprises an amino acid sequence of SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87.

In another aspect, the disclosure provides a fusion protein comprising: (i) the engineered IL-12 p40 polypeptide recited above; and (ii) one or more fusion partners.

15 In certain embodiments, the one or more fusion partners comprise a moiety that reduces aggregation of the engineered IL-12 p40 polypeptide.

In certain embodiments, the one or more fusion partners comprise a moiety that increases expression of the engineered IL-12 p40 polypeptide.

20 In certain embodiments, the one or more fusion partners comprise a moiety that increases serum half-life of the engineered IL-12 p40 polypeptide.

In certain embodiments, the one or more fusion partners comprise a targeting moiety.

In certain embodiments, the one or more fusion partners comprise a serum albumin, polyethylene glycol (PEG), or an antigen binding protein.

25 In certain embodiments, the antigen binding protein binds specifically to urokinase plasminogen activator receptor (uPAR).

In one aspect, the disclosure provides a fusion protein comprising: (i) a urokinase plasminogen activator receptor (uPAR) binding polypeptide; and (ii) a cytokine or variant thereof.

30 In certain embodiments, the uPAR binding polypeptide is linked to the cytokine with an amino acid linker.

In certain embodiments, the cytokine comprises a functional fragment thereof, functional variant thereof or circularly permuted variant thereof.

In certain embodiments, the cytokine is linked via one or more optional amino acid linkers to all or a portion of a receptor for which the cytokine has binding specificity.

5 In certain embodiments, the uPAR binding polypeptide comprises a urokinase plasminogen activator (uPA) polypeptide or variant thereof, capable of specifically binding uPAR.

In certain embodiments, the uPA variant is an amino acid terminal fragment (ATF) of uPA.

10 In certain embodiments, the ATF has the amino acid sequence as set forth in SEQ ID NO: 91, or an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 91.

In certain embodiments, the ATF of uPA comprises one or more amino acid substitutions or deletions.

15 In certain embodiments, the uPA has the amino acid sequence set forth in SEQ ID NO: 90, or an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 90.

In certain embodiments, the cytokine is circularly permuted IL-2 and the receptor is IL-2R α .

20 In certain embodiments, the cytokine is circularly permuted IL-15 and the receptor is IL-15R α .

In certain embodiments, the cytokine is circularly permuted IL-1 and the receptor is IL-1RI, IL-1RII.

25 In certain embodiments, the uPAR binding polypeptide is an anti-uPAR antigen binding protein.

In certain embodiments, the uPAR binding polypeptide is an scFv fragment of an anti-uPAR antibody.

In certain embodiments, the fusion protein comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 98, 99, 100, 106, 107, 111, and 113.

30 In certain embodiments, the fusion protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 98, 99, 111, and 112.

In certain embodiments, the uPAR binding agent, cytokine and receptor are of human origin.

In one aspect, the disclosure provides a method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a fusion protein recited above.

In certain embodiments, the cancer is selected from the group consisting of: melanoma, carcinoma, blastoma and lymphoma.

In one aspect, the disclosure provides a method of treating an inflammatory disease in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a fusion protein recited above.

In certain embodiments, the inflammatory disease is selected from the group consisting of IBD and RA.

In one aspect, the disclosure provides a pharmaceutical composition comprising at least one fusion protein recited above and optionally a pharmaceutically acceptable excipient.

In one aspect, the disclosure provides a fusion protein comprising:
a urokinase plasminogen activator receptor (uPAR) binding polypeptide linked via one or more optional amino acid linkers to:

- (i) a cytokine or variant thereof; or
- (ii) a checkpoint molecule modulator.

In certain embodiments, the cytokine comprises a functional fragment thereof, functional variant thereof or circularly permuted variant thereof.

In certain embodiments, the cytokine is linked via one or more optional amino acid linkers to all or a portion of a receptor for which the cytokine has binding specificity.

In certain embodiments, the uPAR binding polypeptide comprises a urokinase plasminogen activator (uPA) polypeptide or variant thereof, capable of specifically binding uPAR.

In certain embodiments, the uPA variant is an amino acid terminal fragment (ATF) of uPA.

In certain embodiments, the ATF has the amino acid sequence as set forth in SEQ ID NO: 91, or an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 91.

In certain embodiments, the ATF of uPA comprises one or more amino acid substitutions or deletions.

In certain embodiments, the cytokine or checkpoint molecule modulator are selected from the group consisting of: IL-2, circularly permuted IL-2 (cpIL-2), IL-15, circularly permuted IL15 (cpIL-15), IL-6, circularly permuted IL-6 (cpIL-6), IL-10, IL-12, IL-15, GM-CSF, IFN γ , IFN α , 4-1BBB (CD131) or ligand thereof, OX40 or ligand thereof, PD-1, PD-L1, anti-PD-1 antibody, CTLA-4, anti-CTLA4 antibodies, GITR or ligand thereof, ICOS or ligand thereof, CD27, CD70, CD40 or ligand thereof.

In one aspect, the disclosure provides a method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a fusion protein recited above.

In certain embodiments, the cancer is selected from the group consisting of melanoma and lymphoma.

In one aspect, the disclosure provides a method of treating an inflammatory disease in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a fusion protein recited above.

In certain embodiments, the inflammatory disease is selected from the group consisting of IBD and RA.

In one aspect, the disclosure provides a pharmaceutical composition comprising at least one fusion protein recited above and optionally a pharmaceutically acceptable excipient.

In one aspect, the disclosure provides a method of immunotherapy for treating cancer in a patient comprising administering a fusion protein recited above to a patient in need thereof.

In certain embodiments, the method further comprises administering a targeted cancer therapy to the patient.

In certain embodiments, the method further comprises administering a conventional cytotoxic therapy to the patient selected from chemotherapy, radiation therapy or both.

In certain embodiments, the method further comprises administering an immunotherapy selected from a cancer vaccine, a checkpoint inhibitor or a monoclonal antibody.

In certain embodiments, the uPAR binding polypeptide comprises M25 or an amino acid sequence homologous thereto having at least 80% identity at the amino acid level.

In certain embodiments, the uPAR binding polypeptide comprises P25 or an amino acid sequence homologous thereto having at least 80% identity at the amino acid level.

5

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A – FIG. 1B depict anti-uPAR antibody binding data against the DII-DIII domain (**FIG. 1A**) or DIII domain alone (**FIG. 1B**) of human uPAR.

FIG. 2A – FIG. 2B depict anti-uPAR antibody binding data against human (**FIG. 2A**) and mouse (**FIG. 2B**) uPAR expressed on the surface of HEK 293 cells engineered to express human uPAR or mouse uPAR. The anti-uPAR antibodies were incubated with cells over a titration and median fluorescent intensity (MFI) values across the tested antibody concentrations were determined.

FIG. 3 depicts Octet binding data between p35 C74 variants and p40 C177 variants. Along the X-axis, the first amino acid recited for each data point corresponds to the p35 C74 substitution and the second amino acid recited for each data point corresponds to the p40 C177 substitution (i.e., the “K-S” data point is the binding affinity between a p35 C74K variant and a p40 C177S variant).

FIG. 4A – FIG. 4H depict schematics of several exemplary IL-12 subunit fusion proteins. **FIG. 4A** depicts a single chain human IL-12 p35 / p40 fusion protein. **FIG. 4B** depicts a p35 / serum albumin (e.g., human serum albumin or mouse serum albumin) fusion protein. **FIG. 4C** depicts a p35 / Fc CH2-CH3 domain fusion protein paired with a Fab / Fc CH2-CH3 domain fusion protein. Each Fc domain contains a Knob-in-Hole (KiH) mutation to facilitate heterodimerization. **FIG. 4D** depicts a p40 / scFv fusion protein. **FIG. 4E** depicts a p35 / tandem scFv (scFv2) fusion protein. **FIG. 4F** depicts a p35 / Fc CH2-CH3 domain fusion protein paired with a scFv / Fc CH2-CH3 domain fusion protein. Each Fc domain contains a KiH mutation to facilitate heterodimerization. **FIG. 4G** depicts a p40 / Fab domain fusion protein. **FIG. 4H** depicts a p40 / IgG fusion protein. A p40 protein is linked to the C terminus of each CH3 domain of the IgG.

FIG. 5A – FIG. 5B depict tumor and serum pharmacokinetics from PET/CT imaging of radiolabeled tumor-targeted IL-12 subunits p40 (**FIG. 5A**) and p35 (**FIG. 5B**). Tumor-targeted subunits were measured in the tumor and blood of mice over time, and the % of maximum exposure was determined. The tumor targeted p40 was a fusion of the anti-uPAR antibody M37 in a Fab format with p40. The tumor targeted p35 was a fusion of the anti-uPAR antibody M37 in a KiH format with p35.

FIG. 6A – FIG. 6B depict cell-based *in vitro* IL-12 activity data as measured by % Stat4 phosphorylation in CD8+ (**FIG. 6A**) and CD4+ (**FIG. 6B**) T cells. IL-12 subunits were added to T cells at a 1:1 ratio over the recited concentrations.

FIG. 7 depicts cell-based *in vitro* IL-12 activity data as measured by interferon (IFN) gamma secretion by T cells. IL-12 subunits were added to T cells at a 1:1 ratio over the recited concentrations.

FIG. 8A – FIG. 8B depict IL-12 subunit accumulation in the tumor (**FIG. 8A**) and liver (**FIG. 8B**) of mouse tumor models. IL-12 subunit molecules were non-targeted scIL12, targeted Fab-p40, non-targeted p35-MSA, targeted IgG-p40, and targeted p35-KiH. “%ID/g” corresponds to percent of injected dose per gram of tissue.

FIG. 9 depicts serum interferon (IFN) gamma concentration in mice receiving scIL-12 or the combination of p35-MSA and p40-Fab at two doses.

FIG. 10 depicts cell-based *in vitro* IL-12 activity data as measured by % Stat4 phosphorylation in CD8+ T cells in a uPAR-expressing HEK cell / T cell co-culture. IL-12 subunits were added uPAR-expressing HEK cells at a 1:1 ratio over the recited concentrations before co-incubating with the CD8+ T cells.

FIG. 11 depicts accumulation of select fusion proteins in tumor tissue in an RKO xenograft tumor mouse model.

FIG. 12 depicts accumulation of select fusion proteins in serum in an RKO xenograft tumor mouse model.

DETAILED DESCRIPTION

Provided herein are urokinase plasminogen activator receptor (uPAR) binding polypeptides (e.g., uPAR antigen binding proteins), and fusion proteins comprising said uPAR binding polypeptides and a cytokine or variant thereof. The disclosure further provides pharmaceutical compositions comprising the uPAR binding polypeptides and fusion proteins of the disclosure and methods of treating cancer with the same. The disclosure further provides engineered IL-12 p35 and p40 polypeptides.

Generally, nomenclature used in connection with cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein is well-known and commonly used in the art. The methods and techniques provided herein are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein is well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

Unless otherwise defined herein, scientific and technical terms used herein have the meanings that are commonly understood by those of ordinary skill in the art. In the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The use of "or" means "and/or" unless stated otherwise. The use of the term "including," as well as other forms, such as "includes" and "included," is not limiting.

So that the invention may be more readily understood, certain terms are first defined.

Antigen Binding Proteins

As used herein, the term “antibody” or “antigen binding protein” refers to an immunoglobulin molecule or immunoglobulin derived molecule that specifically binds to, or is immunologically reactive with, an antigen or epitope, and includes both polyclonal and monoclonal antibodies, as well as functional antibody fragments, including but not limited to fragment antigen-binding (Fab) fragments, F(ab')₂ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, single chain variable fragments (scFv), and single domain antibodies (e.g., sdAb, sdFv, nanobody, VHH) fragments. The antibody may thus be a single domain antibody or comprise at least one variable light and at least one variable heavy chain.

10 In one embodiment, the at least one variable light and at least one variable heavy chain are linked as a single polypeptide chain. The term “antibody” or “antigen binding protein” includes germline derived antibodies. The term “antibody” or “antigen binding protein” includes genetically engineered or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies,

15 heteroconjugate antibodies (e.g., bispecific antibodies, diabodies, triabodies, tetrabodies, tandem di-scFv, tandem tri-scFv) and the like. Unless otherwise stated, the term “antibody” or “antigen binding protein” should be understood to encompass functional antibody fragments thereof.

In certain embodiments, the antigen binding protein is multispecific (i.e., binds to two or more different target molecules or to two or more epitopes on the same target molecule).

20 In certain embodiments, the antigen binding protein is bispecific and, e.g., binds to two different target molecules or to two epitopes on the same target molecule. In certain embodiments, the antibody is trispecific and e.g., binds to at least three different target molecules.

The antigen binding protein may be monovalent or multivalent, i.e., having one or more antigen binding sites. Non-limiting examples of monovalent antigen binding proteins include scFv, Fab, scFab, dAb, VHH, V(NAR), DARPins, affilins and nanobodies. A multivalent antigen binding protein can have two, three, four or more antigen binding sites. Non-limiting examples of multivalent antigen binding proteins include full-length immunoglobulins, F(ab')₂ fragments, bis-scFv (or tandem scFv or BiTE), DART, diabodies,

30 scDb, DVD-Ig, IgG-scFab, scFab-Fc-scFab, IgG-scFv, scFv-Fc, scFv-fc-scFv, Fv2-Fc,

FynomABs, quadroma, CrossMab, DuoBody, triabodies and tetrabodies. In some embodiments, the multivalent antigen binding protein is bivalent, i.e., two binding sites are present. In some embodiments, the multivalent antigen binding protein is bispecific, i.e., the antigen binding protein is directed against two different targets or two different target sites on one target molecule. In some embodiments, the multivalent antigen binding protein includes more than two, e.g., three or four different binding sites for three or four, respectively, different antigens. Such antigen binding protein is multivalent and multispecific, in particular tri- or tetra- specific, respectively.

As used herein, a “single-chain variable fragment” (scFv) is an antigen binding protein comprising a heavy chain variable domain (VH) linked to a light chain variable domain (VL). The VH and VL domains of the scFv are linked via any appropriate art recognized linker. Such linkers include, but are not limited to, repeated GGGGS amino acid sequences or variants thereof. The scFv is generally free of antibody constant domain regions, although an scFv of the disclosure may be linked or attached to antibody constant domain regions (e.g., antibody Fc domain) to alter various properties of the scFv, including, but not limited to, increased serum or tissue half-life. An scFv generally has a molecular weight of about 25 kDa and a hydrodynamic radius of about 2.5 nm. In certain embodiments, the antigen binding protein comprises tandem scFv, i.e., a first scFv linked to a second scFv. The first and second scFv can be the same amino acid sequence and/or bind to the same epitope. Alternatively, the first and second scFv can be different amino acid sequences and/or bind to different epitopes.

As used herein, a “Fab fragment” or “Fab” is an antibody fragment comprising a light chain fragment comprising a variable light (VL) domain and a constant domain of the light chain (CL), and variable heavy (VH) domain and a first constant domain (CH1) of the heavy chain.

As used herein, a “VHH”, “nanobody”, or “heavy-chain only antibody” is an antigen binding protein comprising a single heavy chain variable domain derived from the species of the Camelidae family, which includes camels, llama, alpaca. A VHH generally has a molecular weight of about 15 kDa.

In one embodiment, the antigen binding protein comprises an Fc domain. The presence of an Fc domain may be advantageous to induce cytotoxic immune responses and/or

activate complement (e.g., ADCC/ADCP or CDC effector function). Exemplary antibody formats including an Fc domain, without being limited to, are full-length immunoglobulins, DVD-Ig, scFv-Fc and scFv-Fc. scFv fusions, IgG-scFab, scFab-dsscFv, Fv2-Fc, IgG-scFv fusions (such as, e.g., bsAb, Bs1Ab, Bs2Ab, Bs3Ab, Ts1Ab, Ts2Ab, Knob-into-Holes (KiHs)), DuoBody and/or CrossMabs. An active Fc domain may increase the likelihood of pro-inflammatory cytokine release by T cells and other effector cells in the tumor microenvironment, which is believed to be part of the therapeutic mechanism of action. The Fc domain may be fully active or partly silenced to avoid over-stimulation of the immune system. In some embodiments, the Fc domain is inactive and does not stimulate pro-inflammatory cytokine release but does still improve half-life and/or stability of the antigen binding protein. In some embodiments, the antigen binding protein comprises a constant region selected from the group consisting of human IgG1, IgG2, IgG3 or IgG4 isotype. In other embodiments, the antigen binding protein comprises a constant region selected from the group consisting of murine IgG1, IgG2A, IgG2B or IgG3 isotype.

The antigen binding proteins of the disclosure may comprise one or more linkers for linking the domains of the antigen binding protein (e.g., linking a VH and VL to form a scFv, or linking multiple binding domains to form a multispecific antigen binding protein).

Illustrative examples of linkers include glycine polymers (Gly)_n; glycine-serine polymers (Gly_nSer)_n, where n is an integer of at least one, two, three, four, five, six, seven, or eight; glycine-alanine polymers; alanine-serine polymers; and other flexible linkers known in the art.

Glycine and glycine-serine polymers are relatively unstructured, and therefore may be able to serve as a neutral tether between domains of fusion proteins such as the antigen binding proteins described herein. Glycine accesses significantly more phi-psi space than other small side chain amino acids, and is much less restricted than residues with longer side chains (Scheraga, Rev. Computational Chem. 1: 1173-142 (1992)). A person skilled in the art will recognize that design of an antigen binding protein in particular embodiments can include linkers that are all or partially flexible, such that the linker can include flexible linker stretches as well as one or more stretches that confer less flexibility to provide a desired structure.

Linker sequences can, however, be chosen to resemble natural linker sequences, for example, using the amino acid stretches corresponding to the beginning of human CH1 and C κ sequences or amino acid stretches corresponding to the lower portion of the hinge region of human IgG.

5 The design of the peptide linkers connecting VL and VH domains in the scFv moieties are flexible linkers generally composed of small, non-polar or polar residues such as, e.g., Gly, Ser and Thr. A particularly exemplary linker connecting the variable domains of the scFv moieties is the (Gly₄Ser)₄ linker, where 4 is the exemplary number of repeats of the motif. In certain embodiments, the linker comprises the amino acid sequence
 10 GGSGGGSGGGSGGGSGGGSGGGSGG, GGGGSGGGSGGGGS, or GGGSGGGSG (SEQ ID NO: 246).

In certain embodiments, the amino acid linker comprises (GGGS)_n (SEQ ID NO: 148), wherein n is an integer between 1 and 5. In certain embodiments, the amino acid linker comprises (GGGS)_n (SEQ ID NO: 247), wherein n is an integer between 1 and 5.

15 Other exemplary linkers include, but are not limited to the following amino acid sequences: GGG; DGGS; TGEKP (Liu et al., Proc. Natl. Acad. Sci.94: 5525-5530 (1997)); GGRR; (GGGS)_n (SEQ ID NO: 148) wherein n = 1, 2, 3, 4 or 5 (Kim et al, Proc. Natl. Acad. Sci.93: 1156-1160 (1996)); EGKSSGSGSESKVD (Chaudhary et al., Proc. Natl. Acad. Sci. 87: 1066-1070 (1990)); KESGSVSSEQLAQFRSLD (Bird et al., Science 242:423- 426
 20 (1988)), GGRRGGGS; LRQRDGERP; LRQKDGGSERP; and GSTSGSGKPGSGEGSTKG (Cooper et al., Blood, 101(4): 1637-1644 (2003)). Alternatively, flexible linkers can be rationally designed using a computer program capable of modeling the 3D structure of proteins and peptides or by phage display methods.

The antibodies may comprise a variable light (VL) domain and a variable heavy (VH)
 25 domain. Each VL and VH domain further comprises a set of three CDRs.

As used herein, the term “complementarity determining region” or “CDR” refers to non-contiguous sequences of amino acids within antibody variable regions, which confer antigen specificity and binding affinity. In general, there are three CDRs in each heavy chain variable domain (HCDR1, HCDR2, HCDR3) and three CDRs in each light chain variable
 30 domain (LCDR1, LCDR2, LCDR3). “Framework regions” or “FRs” are known in the art to

refer to the non-CDR portions of the variable domains of the heavy and light chains. In general, there are four FRs in each heavy chain variable domain (HFR1, HFR2, HFR3, and HFR4), and four FRs in each light chain variable domain (LFR1, LFR2, LFR3, and LFR4). Accordingly, an antibody variable region amino acid sequence can be represented by the formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. Each segment of the formula, i.e., FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4, represents a discrete amino acid sequence (or a polynucleotide sequence encoding the same) that can be mutated, including one or more amino acid substitutions, deletions, and insertions. In certain embodiments, an antibody variable light chain amino acid sequence can be represented by the formula LFR1-LCDR1-LFR2-LCDR2-LFR3-LCDR3-LFR4. In certain embodiments, an antibody variable heavy chain amino acid sequence can be represented by the formula HFR1-HCDR1-HFR2-HCDR2-HFR3-HCDR3-HFR4.

The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. ("Kabat" numbering scheme), Al-Lazikani et al., (1997) JMB 273, 927-948 ("Chothia" numbering scheme), MacCallum et al., J. Mol. Biol. 262:732-745 (1996), "Antibody-antigen interactions: Contact analysis and binding site topography," J. Mol. Biol. 262, 732-745. ("Contact" numbering scheme), Lefranc M P et al., "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev Comp Immunol, 2003 January; 27(1):55-77 ("IMGT" numbering scheme), and Honegger A and Pluckthun A, "Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool," J Mol Biol, 2001 Jun. 8; 309(3):657-70, ("AHO" numbering scheme).

The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based structural alignments, while the Chothia scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions accommodated by insertion letters, for example, "30a," and deletions appearing in some antibodies. The two schemes place certain insertions and deletions ("indels") at different

positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme.

Thus, unless otherwise specified, a “CDR” or “complementary determining region,” or individual specified CDRs (e.g., HCDR1, HCDR2), of a given antibody or fragment thereof, such as a variable domain thereof, should be understood to encompass a (or the specific) complementary determining region as defined by any of the known schemes. Likewise, unless otherwise specified, an “FR” or “framework region,” or individual specified FRs (e.g., “HFR1,” “HFR2”) of a given antibody or fragment thereof, such as a variable domain thereof, should be understood to encompass a (or the specific) framework region as defined by any of the known schemes. In some instances, the scheme for identification of a particular CDR or FR is specified, such as the CDR as defined by the Kabat, Chothia, or Contact method. In other cases, the particular amino acid sequence of a CDR or FR is given.

In certain embodiments, the antigen binding proteins disclosed here are humanized. As used herein, the term “humanized” or “humanization” refers to an antigen binding protein that has been altered to make it more like a human antibody. Non-human antigen binding proteins, such as the murine antigen binding proteins disclosed herein, would elicit a negative immune reaction if administered to a human for therapy. It is therefore advantageous to humanize the non-human (e.g., murine) antigen binding proteins for later therapeutic use.

In certain embodiments, the antigen binding proteins are humanized through resurfacing (i.e., remodel the solvent-accessible residues of the non-human framework such that they become more human-like). Resurfacing strategies are described in more detail in WO2004/016740, WO2008/144757, and WO2005/016950, each of which is incorporated herein by reference.

In certain embodiments, the antigen binding proteins are humanized through CDR grafting (i.e., inserting the murine antigen binding protein CDRs into a human antibody acceptor framework).

As used herein, the term “affinity” refers to the strength of the interaction between an antibody’s antigen binding site and the epitope to which it binds. As readily understood by those skilled in the art, an antibody or antigen binding protein affinity may be reported as a dissociation constant (KD) in molarity (M). The antibodies of the disclosure may have KD

values in the range of 10^{-8} to 10^{-14} M. High affinity antibodies have K_D values of 10^{-9} M (1 nanomolar, nM) and lower. For example, a high affinity antibody may have a K_D value in the range of about 1 nM to about 0.01 nM. A high affinity antibody may have K_D value of about 1 nM, about 0.9 nM, about 0.8 nM, about 0.7 nM, about 0.6 nM, about 0.5 nM, about 0.4 nM, about 0.3 nM, about 0.2 nM, or about 0.1 nM. Very high affinity antibodies have K_D values of 10^{-12} M (1 picomolar, pM) and lower. Weak, or low, affinity antibodies may have K_D values in the range of 10^{-1} to 10^{-4} M. Low affinity antibodies may have K_D values of 10^{-4} M and higher, such as 10^{-4} M, 10^{-3} M, 10^{-2} M, or 10^{-1} M.

The ability of an antibody to bind to a specific antigenic determinant (e.g., uPAR) can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g., surface plasmon resonance (SPR) technique (analyzed on a BIAcore instrument) (Liljeblad et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Heeley, Endocr Res 28, 217-229 (2002)).

Various antibody constant domain amino acid sequences used in the disclosure are recited below in **Table 1**.

Table 1 – Antibody Constant Domain Amino Acid Sequences

Sequence ID	Sequence
IgG1 CH1-hinge-CH2-CH3 (WT)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSGDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHY TQKSLSLSPGK (SEQ ID NO: 72)
IgG1 CH1-hinge-CH2-CH3 (LALA)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNH YTQKSLSLSPGK (SEQ ID NO: 73)
IgG1 CH1-hinge-CH2-	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS

CH3 (LALA-PG)	WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTP PVLDSGDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHN HYTQKSLSLSPGK (SEQ ID NO: 74)
IgG1 CH2-CH3 (T366W mutation - Knob)	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT EVTCTVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVCTLPSSREEMTKNQVSLWCLVKGFY PSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDK SRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 75)
IgG1 CH2-CH3 (T366S, L368A, Y407V mutations – Hole)	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT EVTCTVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVCTLPSSREEMTKNQVSLSCAVKGFY PSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDK SRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 76)
IgG1 CH2-CH3 (Knob T366W; LALA-PG)	EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT EVTCTVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVCTLPSSREEMTKNQVSLWCLVKGFY PSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDK SRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 77)
IgG1 CH2-CH3 (Hole T366S, L368A, Y407V; LALA-PG; H435R; Y436F)	EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT EVTCTVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPCREEMTKNQVSLSCAVKGFY PSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDK SRWQQGNV FSCSVMHEALHNRF TQKSLSLSPGK (SEQ ID NO: 78)
IgG1 CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHNKPSNTKVDKKVEPKSC (SEQ ID NO: 79)
Kappa CL1	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 80)

Antigen Binding Protein Heterodimerization

In an aspect of the disclosure, antigen binding proteins comprising two Fc domains are heterodimerized through knobs-into-holes pairing (KiH). This dimerization technique utilizes protuberances (“knobs”) and cavities (“holes”) engineered into the interface of CH3 domains. Where a suitably positioned and dimensioned knob or hole exists at the interface of either the first or second CH3 domain, it is only necessary to engineer a corresponding hole or knob, respectively, at the adjacent interface, thus promoting and strengthening Fc domain pairing in the CH3/CH3 domain interface. A “knob” refers to an at least one amino acid side chain, typically a larger side chain, that protrudes from the interface of the CH3 portion of a first Fc domain. The protrusion creates a “knob” which is complementary to and received by a “hole” in the CH3 portion of a second Fc domain. The “hole” is an at least one amino acid side chain, typically a smaller side chain, which recedes from the interface of the CH3 portion of the second Fc domain. This technology is described, for example, in US Patent No. 5,821,333; Ridgway et al., Protein Engineering 9:617-621 (1996); and Carter P., J. Immunol. Methods 248: 7-15 (2001).

Exemplary amino acid residues that may act as a knob include arginine (R), phenylalanine (F), tyrosine (Y), and tryptophan (W). An existing amino acid residue in the CH3 domain may be replaced or substituted with a knob amino acid residue. Preferred amino acids to be substituted may include any amino acid with a small side chain, such as alanine (A), asparagine (N), aspartic acid (D), glycine (G), serine (S), threonine (T), or valine (V).

Exemplary amino acid residues that may act as a hole include alanine (A), serine (S), threonine (T), and valine (V). An existing amino acid residue in the CH3 domain may be replaced or substituted with a hole amino acid residue. Preferred amino acids to be substituted may include any amino acid with a large or bulky side chain, such as arginine (R), phenylalanine (F), tyrosine (Y), or tryptophan (W).

The CH3 domain can be derived from a human IgG1 antibody. Exemplary amino acid substitutions to the CH3 domain include T366Y, T366W, F405A, F405W, Y407T, Y407A, Y407V, T394S, and combinations thereof. A certain exemplary combination is T366Y or T366W for the knob mutation on a first CH3 domain and Y407T or Y407V for the hole mutation on a second CH3 domain.

In certain embodiments, the KiH Fc domain comprises a knob Fc portion comprising the amino acid substitution T366W and a hole Fc portion comprising the amino acid

substitutions T366S, L368A, Y407V, according to Eu numbering. In certain embodiments, the hole Fc portion further comprises the amino acid substitutions H435R and Y436F, according to Eu numbering.

5 In certain embodiments of the disclosure, antigen binding proteins comprising two Fc domains are heterodimerized through Fab arm exchange (FAE). A human IgG1 possessing a P228S hinge mutation may contain an F405L or K409R CH3 domain mutation. Mixing of the two antibodies with a reducing agent leads to FAE. This technology is described in US Patent No. 9,212,230 and Labrijn A.F., Proc Natl Acad Sci USA 110(13): 5145-5150 (2013).

10 In certain embodiments of the disclosure, antigen binding proteins comprising two Fc domains are heterodimerized through electrostatic steering effects. This dimerization technique utilizes electrostatic steering to promote and strengthen Fc domain pairing in the CH3/CH3 domain interface. The charge complementarity between two CH3 domains is altered to favor heterodimerization (opposite charge pairing) over homodimerization (same charge pairing). In this method, the electrostatic repulsive forces prevent homodimerization.
15 Exemplary amino acid residue substitutions may include K409D, K392D, and/or K370D in a first CH3 domain, and D399K, E356K, and/or E357K in a second CH3 domain. This technology is described in US Patent Application Publication No. 2014/0154254 A1 and Gunasekaran K., J Biol Chem 285(25): 19637-19646 (2010).

20 In certain embodiments of the disclosure, antigen binding proteins comprising two Fc domains are heterodimerized through hydrophobic interaction effects. This dimerization technique utilizes hydrophobic interactions instead of electrostatic ones to promote and strengthen Fc domain pairing in the CH3/CH3 domain interface. Exemplary amino acid residue substitution may include K409W, K360E, Q347E, Y349S, and/or S354C in a first CH3 domain, and D399V, F405T, Q347R, E357W, and/or Y349C in a second CH3 domain.
25 Preferred pairs of amino acid residue substitutions between a first CH3 domain and a second CH3 domain include K409W:D399V, K409W:F405T, K360E:Q347R, Y349S:E357W, and S354C:Y349C. This technology is described in US Patent Application Publication No. 2015/0307628 A1.

30 In certain embodiments of the disclosure, antigen binding proteins comprising two Fc domains are heterodimerized through the use of leucine zipper fusions. Leucine zipper domains fused to the C terminus of each CH3 domain of the antibody chains force

heterodimerization. This technology is described in Wranik B., J Biol Chem 287(52): 43331-43339 (2012).

In certain embodiments of the disclosure, antigen binding proteins comprising two Fc domains are heterodimerized through the use of a Strand Exchange Engineered Domain (SEED) body. CH3 domains derived from an IgG and IgA format force heterodimerization. This technology is described in Muda M., Protein Eng. Des. Sel. 24(5): 447-454 (2011).

Unless otherwise stated, all antibody constant region numbering employed herein corresponds to the EU numbering scheme, as described in Edelman et al., Proc. Natl. Acad. Sci. USA 63(1): 78-85 (1969).

Additional methods of heterodimerization of heavy and/or light chains and the generation and purification of asymmetric antibodies are known in the art. See, for example, Klein C., mABs 4(6): 653-663 (2012), and U.S. Patent No. 9,499,634, each of which is incorporated herein by reference.

Additional Fc domain mutations are envisioned to alter Fc function, including, but not limited to, altered Antibody Dependent Cellular Cytotoxicity (ADCC) and Complement-Dependent Cytotoxicity (CDC).

In certain embodiments, the antigen binding protein comprises a IgG1 constant domain with a L234A and L235A amino acid substitution, according to Eu numbering.

In certain embodiments, the antigen binding protein comprises a IgG1 constant domain with a P329G amino acid substitution, according to Eu numbering.

Urokinase Plasminogen Activator Receptor (uPAR) Binding Polypeptides

As used herein, the term “uPAR” or “urokinase plasminogen activator receptor” or “urokinase receptor” or “uPA receptor” or “CD87” or “Cluster of Differentiation 87” refers to the target of the antigen binding proteins described herein. uPAR is composed of three different domains of the Ly-6/uPAR/alpha-neuro toxin family. All three domains are involved in high affinity binding of the primary ligand, urokinase. Besides the primary ligand urokinase, uPAR interacts with several other proteins, including vitronectin, the uPAR associated protein (uPARAP), and the integrin family of membrane proteins.

As used herein, the term “binding polypeptide” or “binding protein” corresponds to a polypeptide that specifically binds to a target protein (e.g., uPAR). Binding polypeptides include, but are not limited to, antigen binding proteins, as described above, as well as ligands to the target protein. In certain embodiments, the binding polypeptide is a urokinase plasminogen activator (uPA) polypeptide or variant thereof, capable of specifically binding uPAR. In certain embodiments, the uPA variant is an amino acid terminal fragment (ATF) of uPA. In certain embodiments, the ATF has the amino acid sequence as set forth in SEQ ID NO: 91, or an amino acid sequence with at least 80% identity (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) to the amino acid sequence as set forth in SEQ ID NO: 91. In certain embodiments, the ATF of uPA comprises one or more amino acid substitutions or deletions. In certain embodiments, the uPA has the amino acid sequence set forth in SEQ ID NO: 90, or an amino acid sequence with at least 80% identity (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) to the amino acid sequence as set forth in SEQ ID NO: 90.

In one aspect, the disclosure provides an isolated antigen binding protein that binds specifically to urokinase plasminogen activator receptor (uPAR), comprising:

(a) an antibody heavy chain variable (VH) domain comprising:

i) an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), GFSLTNYG (SEQ ID NO: 11), GNTFTDYG (SEQ ID NO: 19), GTTFTDYG (SEQ ID NO: 41), or GYTFTSYG (SEQ ID NO: 27);

ii) an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), IWSDGGT (SEQ ID NO: 12), INTNTGEP (SEQ ID NO: 20), or IYPRSGNT (SEQ ID NO: 28); and

iii) an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5), ARGGRSDFAY (SEQ ID NO: 13), AHYSFDY (SEQ ID NO: 21), or AGKDYGSTYADY (SEQ ID NO: 29); and

(b) an antibody light chain variable (VL) domain comprising:

iv) an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), QSIVHSNGNTY (SEQ ID NO: 14), ENIYSN (SEQ ID NO: 22), SSVSSRY (SEQ ID NO: 30);

v) an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), KVS (SEQ ID NO: 15), AAT (SEQ ID NO: 23), or GTS (SEQ ID NO: 31); and

vi) an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8), FQGSHVPYT (SEQ ID NO: 16), QHFWGTPWT (SEQ ID NO: 24), QQYHSDPLT (SEQ ID NO: 32).

5 In certain embodiments, the antigen binding proteins of the disclosure comprise at least about 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence similarity or identity to any of the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, or LCDR3 amino acid sequences as set forth above and in **Table 3** and **Table 8**.

10 In certain embodiments, the VH domain comprises an amino acid sequence of SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 37, SEQ ID NO: 42, or SEQ ID NO: 43, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, or SEQ ID NO: 44.

15 In certain embodiments, the antigen binding proteins of the disclosure comprise at least about 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence similarity or identity to any of the VH or VL amino acid sequences as set forth above and in **Table 3** and **Table 8**.

In certain embodiments:

- 20 a) the VH domain comprises an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5); and
- b) the VL domain comprises an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), and an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8).

In certain embodiments:

- 25 a) the VH domain comprises an HCDR1 amino acid sequence of GFSLTNYG (SEQ ID NO: 11), an HCDR2 amino acid sequence of IWSGGT (SEQ ID NO: 12), an HCDR3 amino acid sequence of ARGGRSDLFAY (SEQ ID NO: 13); and
- b) the VL domain comprises an LCDR1 amino acid sequence of QSIVHSNGNTY (SEQ ID NO: 14), an LCDR2 amino acid sequence of KVS (SEQ ID NO: 15), and an LCDR3 amino acid sequence of FQGSHVPYT (SEQ ID NO: 16).

30 In certain embodiments:

a) the VH domain comprises an HCDR1 amino acid sequence of GNTFTDYG (SEQ ID NO: 19), or GTTFTDYG (SEQ ID NO: 41), an HCDR2 amino acid sequence of INTNTGEP (SEQ ID NO: 20), an HCDR3 amino acid sequence of AHYSFDY (SEQ ID NO: 21); and

- 5 b) the VL domain comprises an LCDR1 amino acid sequence of ENIYSN (SEQ ID NO: 22), an LCDR2 amino acid sequence of AAT (SEQ ID NO: 23), and an LCDR3 amino acid sequence of QHFWGTPWT (SEQ ID NO: 24).

In certain embodiments:

- 10 a) the VH domain comprises an HCDR1 amino acid sequence of GYTFTSYG (SEQ ID NO: 27), an HCDR2 amino acid sequence of IYPRSGNT (SEQ ID NO: 28), an HCDR3 amino acid sequence of AGKDYGSTYADY (SEQ ID NO: 29); and

 b) the VL domain comprises an LCDR1 amino acid sequence of SSVSSRY (SEQ ID NO: 30), an LCDR2 amino acid sequence of GTS (SEQ ID NO: 31), and an LCDR3 amino acid sequence of QQYHSDPLT (SEQ ID NO: 32).

- 15 In certain embodiments:

 a) the VH domain comprises an amino acid sequence of SEQ ID NO: 33, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35;

 b) the VH domain comprises an amino acid sequence of SEQ ID NO: 33, and the VL domain comprises an amino acid sequence of SEQ ID NO: 36;

- 20 c) the VH domain comprises an amino acid sequence of SEQ ID NO: 34, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35;

 d) the VH domain comprises an amino acid sequence of SEQ ID NO: 34, and the VL domain comprises an amino acid sequence of SEQ ID NO: 36;

- 25 e) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 38;

 f) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 39;

 g) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 40;

- 30 h) the VH domain comprises an amino acid sequence of SEQ ID NO: 42, and the VL domain comprises an amino acid sequence of SEQ ID NO: 44; or

i) the VH domain comprises an amino acid sequence of SEQ ID NO: 43, and the VL domain comprises an amino acid sequence of SEQ ID NO: 44.

In certain embodiments, the VH domain and VL domain are attached with an amino acid linker.

5 In certain embodiments, the amino acid linker comprises (GGGGS)_n (SEQ ID NO: 148), wherein n is an integer between 1 and 5.

In certain embodiments, the amino acid linker comprises the amino acid sequence GGGGSGGGSGGGGS (SEQ ID NO: 149) or GGS GGGGSGGGSGGGSGGGSGGGSGG (SEQ ID NO: 150).

10 In certain embodiments, the antigen binding protein comprises a Fab fragment, a F(ab')₂ fragment, an Fd fragment, an Fv fragment, a single chain Fv (scFv), a dAb fragment, a single domain antibody, or a nanobody.

In certain embodiments, the antigen binding protein comprises the amino acid sequence of any one of SEQ ID NOs: 56-71.

15 In certain embodiments, the antigen binding protein further comprises the amino acid sequence of any one of SEQ ID NOs: 72-78.

In certain embodiments, the antigen binding protein comprises an antibody heavy chain (HC) amino acid sequence of SEQ ID NO: 45, 46, 49, 53, or 54, and an antibody light chain (LC) amino acid sequence of SEQ ID NO: 47, 48, 50, 51, 52, and 55.

20 In certain embodiments:

a) the antibody HC comprises the amino acid sequence of SEQ ID NO: 45, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 47;

b) the antibody HC comprises the amino acid sequence of SEQ ID NO: 45, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 48;

25 c) the antibody HC comprises the amino acid sequence of SEQ ID NO: 46, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 47;

d) the antibody HC comprises the amino acid sequence of SEQ ID NO: 46, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 48;

30 e) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 50;

f) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 51;

g) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 52;

5 h) the antibody HC comprises the amino acid sequence of SEQ ID NO: 53, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 55; or

i) the antibody HC comprises the amino acid sequence of SEQ ID NO: 54, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 55.

In certain embodiments, the antigen binding protein binds human uPAR with a KD of
10 less than about 5×10^{-8} M, less than about 1×10^{-8} M, less than about 5×10^{-9} M, less than about 1×10^{-9} M, less than about 5×10^{-10} M, or less than about 1×10^{-10} M.

In certain embodiments, the antigen binding protein binds human uPAR in the presence of a uPAR ligand.

In certain embodiments, the antigen binding protein thereof binds uPAR ligand-bound
15 human uPAR.

In certain embodiments, the antigen binding protein binds human uPAR on human uPAR-expressing cells, thereby producing a fluorescent signal detected with a fluorescently-labelled antibody that binds to the antigen binding protein, with an EC50 value of about 0.1 nM to about 3.0 nM.

20 In certain embodiments, the antigen binding protein binds human uPAR on human uPAR-expressing cells in the presence of a uPAR ligand.

In certain embodiments, the antigen binding protein binds a human uPAR DII-DIII domain or a human uPAR DIII domain.

In one aspect, the disclosure provides an isolated antigen binding protein that
25 competes for binding with the antigen binding protein described above.

In one aspect, the disclosure provides an isolated antigen binding protein that binds the same epitope as the antigen binding protein described above.

In one aspect, the disclosure provides a pharmaceutical composition comprising the antigen binding protein described above, and a pharmaceutically acceptable carrier or diluent.

30 In one aspect, the disclosure provides an isolated nucleic acid molecule comprising a polynucleotide sequence that encodes the antigen binding protein described above.

In one aspect, the disclosure provides an expression vector comprising the polynucleotide sequence described above.

In one aspect, the disclosure provides a host cell comprising the expression vector described above.

5 In another aspect, the disclosure provides a method of manufacturing the antigen binding protein recited above, comprising the steps of:

(i) cultivating the host cell recited above under conditions allowing expression of the antigen binding protein;

(ii) recovering the antigen binding protein; and optionally

10 (iii) further purifying and/or modifying and/or formulating the antigen binding protein.

Cytokines And Variants Thereof

As used herein, a “cytokine” is a general term that includes “lymphokine” (cytokines made by lymphocytes), “monokine” (cytokines made by monocytes), “chemokine” (cytokines with chemotactic activities), and “interleukin” (cytokines made by one leukocyte and acting on other leukocytes). Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action). Pro-inflammatory cytokines are produced predominantly by activated macrophages and dendritic cells and are involved in the up-regulation of inflammatory reactions and include, but are not limited to IL-1 β , IL-6, and TNF- α . Chemokines are usually assigned to 4 groups depending on the spacing of their first two cysteine residues: C-C chemokines (e.g. RANTES, monocyte chemoattractant protein or MCP-1, monocyte inflammatory protein or MIP-1 α , and MIP-1 β), C-X-C chemokines (e.g. IL-8 also called growth related oncogene or GRO/KC), C chemokines (e.g. lymphotactin), and CXXXC chemokines (e.g. fractalkine). Anti-inflammatory cytokines are a series of immunoregulatory molecules that dampen or control the pro-inflammatory response. Cytokines act in concert with specific cytokine inhibitors and soluble cytokine receptors to regulate immune responses. Major anti-inflammatory cytokines include, but are not limited to: interleukin (IL)-1 receptor antagonist, IL-4, IL-10, IL-11, and IL-13. Leukemia inhibitory factor, interferon-alpha, IL-6, IL-10 and transforming growth factor (TGF)- β are categorized as either anti-inflammatory or pro-inflammatory cytokines,

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under various circumstances. Specific cytokine receptors for IL-1, TNF- α , and IL-18 also function as inhibitors for pro-inflammatory cytokines.

The cytokine polypeptide of the fusion proteins disclosed herein can be any cytokine, including variants thereof, such as a functional fragment thereof, or a circularly permuted variant thereof. Useful cytokines include but are not limited to: IL-1 α , IL-1 β , IL-1Ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-12p35, IL-12p40, IL-13, IL-15, IL-17 family members, IL-18, IL-21, IL-22, IL-23, IL-23p19, IL-30 (IL-27p28), IL-33, IL-34, IL-35, IL-35p35, IL-36Ra, IL-36a, IL-36b, IL-36g, IL-37, IL-38, LIF, CNTF, Oncostatin M, CLCF-1, GM-CSF, Oxferritin, apolipoprotein e, tumor necrosis factor α (TNF α) interferon-alpha (IFN α), interferon-beta (IFN β), or interferon-gamma (IFN γ). In certain embodiments, the cytokine or variant thereof is selected from the group consisting of IL-2, circularly permuted IL-2 (cpIL-2), IL-15, circularly permuted IL15 (cpIL-15), IL-6, circularly permuted IL-6 (cpIL-6), IL-10, or IL-12. In certain embodiments, the cytokine or variant thereof comprises at least one interleukin-12 (IL-12) subunit (e.g., p35 and p40). In certain embodiments, the IL-12 subunit comprises one or both of p35 and p40. In certain embodiments, the cytokine or variant thereof comprises a single-chain IL-12 (scIL-12) comprising a p35 polypeptide and a p40 polypeptide attached by an amino acid linker.

As used herein, the term “variant” refers to a polypeptide that differs from a reference polypeptide, but retains essential properties. A typical variant of a polypeptide differs in its primary amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more modifications (e.g., substitutions, additions, and/or deletions). A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polypeptide may be naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. In addition, the term “variant” as used herein includes circular permutations of proteins and peptides.

As used herein, the terms “circular permutation” and “circularly permuted” “(CP or cp)”, refers to the conceptual process of taking a linear protein, or its cognate nucleic acid sequence, and fusing the native N- and C-termini (directly or through a linker, using protein or recombinant DNA methodologies) to form a circular molecule, and then cutting (opening)

the circular molecule at a different location to form a new linear protein, or cognate nucleic acid molecule, with termini different from the termini in the original molecule. Circular permutation thus preserves the sequence, structure, and function of a protein (other than the optional linker), while generating new C- and N-termini at different locations that, in accordance with one aspect of the disclosure, results in an improved orientation for fusing a desired polypeptide fusion partner as compared to the original ligand. Circular permutation also includes any process that results in a circularly permuted straight-chain molecule, as defined herein. In general, a circularly permuted molecule is *de novo* expressed as a linear molecule and does not formally go through the circularization and opening steps. The particular circular permutation of a molecule, herein, is designated by brackets containing, in the case of a circularly permuted protein, the amino acid residues between which the peptide bond is eliminated. For example, the designation IL6(Q182/Q180) designates a circularly permuted IL6 growth factor in which the opening site (position at which the peptide bond is eliminated) occurred between residues Q182 and Q180 of the unpermuted or unmodified native IL6, and thus the newly created N-terminus is a Glutamine which was formerly residue 182, and the newly created C-terminus is a Glutamine which was formerly residue 180. Circularly permuted proteins, and methods of producing the same, are described in more detail in U.S. 9,359,415, incorporated herein by reference.

The terms “unpermuted,” “native,” “wild type,” or “unmodified” ligand, polypeptide, protein, cytokine, for example, are used herein to provide a reference point for the cytokine, prior to its rearrangement into a circularly permuted molecule, as described above. Typically, the unmodified cytokine has amino and carboxy termini and an amino acid sequence that correspond substantially to the amino and carboxy termini and amino acid sequence of the cytokine or an independent domain of a protein, as it generally occurs *in vivo*. The unmodified cytokine may be a fully mature form or a precursor to the mature form (such as a pro-protein).

As used herein, a “fragment” refers to a segment of the polypeptide that is shorter than the reference protein. Fragments of a protein can have terminal (carboxy or amino- terminal) and/or internal deletions. Generally, fragments of a protein will be at least four (e.g., at least five, at least six, at least seven, at least eight, at least nine, at least 10, at least 12, at least 15, at least 18, at least 25, at least 30, at least 35, at least 40, at least 50, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, or at least 100 or more) amino acids in

length. Biologically active fragments or biologically active variants of any of the proteins, fusion proteins or fragments described herein have at least 25% (e.g., at least: 30%; 40%; 50%; 60%; 70%; 75%; 80%; 85%; 90%; 95%; 97%; 98%; 99%; 99.5%, or 100% or even greater) of the activity of the wild-type, full-length reference protein. In the case of a uPAR binding polypeptide, the relevant activity is the ability of the uPAR binding polypeptide is to bind to the target uPAR receptor. In the case of the cytokine polypeptide, the relevant activity is the ability bind its receptor and agonize or antagonize appropriately. Depending on their intended use, the polypeptides, biologically active fragments, or biologically active variants thereof can be of any species, but is preferably a mammal (e.g., a mouse, rat, rabbit, hamster, gerbil, dog, cat, goat, pig, cow, horse, whale, monkey, or human) and most preferably a human.

It is therefore understood that the disclosure provides (i) biologically active variants and (ii) biologically active fragments or biologically active variants thereof, of the full-length polypeptides (e.g., the various polypeptides of the fusion proteins of the invention including the uPAR binding polypeptide, cytokine and corresponding receptor or receptor fragment as described herein. Biologically active variants of full-length, preferably mature, wild-type proteins or fragments of the proteins can contain additions, deletions, or substitutions such as one, two, three, four, five, six, seven, eight, nine, ten, 12, 15, 20, 25, 30, 35, 40, or 50 conservative amino acid substitutions. A conservative substitution is the substitution of one amino acid for another with similar characteristics. Conservative substitutions include substitutions within the following groups: valine, alanine and glycine; leucine, valine, and isoleucine; aspartic acid and glutamic acid; asparagine and glutamine; serine, cysteine, and threonine; lysine and arginine; and phenylalanine and tyrosine. The non- polar hydrophobic amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Any substitution of one member of the above-mentioned polar, basic or acidic groups by another member of the same group can be deemed a conservative substitution. By contrast, a non-conservative substitution is a substitution of one amino acid for another with dissimilar characteristics. Deletion variants can lack one, two, three, four,

five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid segments (of two or more amino acids) or noncontiguous single amino acids. Additions (addition variants) include fusion proteins containing: (a) full-length, wild-type polypeptides or fragments thereof containing at least five amino acids; and (b) internal or terminal (C or N) irrelevant or heterologous amino acid sequences. In the context of such fusion proteins, the term “heterologous amino acid sequences” refers to an amino acid sequence other than (a). A fusion protein containing a peptide described herein and a heterologous amino acid sequence thus does not correspond in sequence to all or part of a naturally occurring protein. A heterologous sequence can be, for example a sequence used for purification of the recombinant protein (e.g., FLAG or polyhistidine (hexahistidine or 6xHis)).

Engineered Interleukin 12 Subunits

IL-12 is a heterodimer, composed of a p35 subunit (i.e., IL-12 p35) and a p40 subunit (i.e., IL-12 p40). Optimal activity of IL-12 is achieved when the heterodimer forms and binds to the IL-12 receptor (e.g., a IL-12R β 1 / IL-12R β 2 heterodimer). The two subunits (p35 and p40) can be linked together to form a single chain IL-12 (scIL-12), thereby eliminating the need for heterodimer assembly and increasing the activity of IL-12. However, scIL-12 is highly toxic when administered systemically. It is therefore advantageous to supply each IL-12 subunit separately.

Unfortunately, the IL-12 subunits are difficult to express and purify for therapeutic purposes. p35 shows little to no expression. Expression can be improved by linking p35 to a fusion partner, such as an antibody or fragment thereof, or human serum albumin (HSA). p35 is also prone to aggregation. p40 shows similar challenges. p40 can be expressed without a fusion partner, but the purified composition is frequently contaminated with a p40 homodimer (about 15-20%). p40 is also prone to dimerization and aggregation over time at 37 °C. The instant disclosure provides engineered IL-12 subunits (i.e., p35 and p40 variants) that can overcome these challenges while retaining binding affinity of each other.

In one aspect, the disclosure provides an engineered IL-12 p35 polypeptide, comprising one or more amino acid substitutions at positions S27, N28, Q35, F39, S44, C74, M111, V114, M119, M145, F150, and L161 of SEQ ID NO: 81.

In certain embodiments, the engineered IL-12 p35 polypeptide further comprises one or both of a S27 and D188 amino acid substitution.

In certain embodiments, the amino acid substitution comprises M12S; S27D; N28R; Q35E; F39D or F39H; S44K; C74K, C74D, C74W, C74Y, or C74Q; M111F or M111W;
5 V114I; M119L; M145L; F150Y; L161D or L161Q; D188N; or a combination thereof.

In certain embodiments, the amino acid substitution comprises N28R and Q35E.

In certain embodiments, the amino acid substitution comprises C74K.

In certain embodiments, the amino acid substitution comprises S27D, C74K, and D188N.

10 In certain embodiments, the engineered IL-12 p35 polypeptide comprises an amino acid sequence of SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83, or an amino acid sequence with at least about 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence similarity or identity to the amino acid sequences as set forth in SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.

15 In a further aspect, the disclosure provides a fusion protein comprising: (i) the engineered IL-12 p35 polypeptide recited above; and (ii) one or more fusion partners (e.g., a functional moiety or anti-uPAR antigen binding protein).

In certain embodiments, the one or more fusion partners comprise a moiety that reduces aggregation of the engineered IL-12 p35 polypeptide.

20 In certain embodiments, the one or more fusion partners comprise a moiety that increases expression of the engineered IL-12 p35 polypeptide.

In certain embodiments, the one or more fusion partners comprise a moiety that increases serum half-life of the engineered IL-12 p35 polypeptide.

In certain embodiments, the one or more fusion partners comprise a targeting moiety.

25 In certain embodiments, the one or more fusion partners comprise a serum albumin, polyethylene glycol (PEG), or an antigen binding protein.

In certain embodiments, the antigen binding protein binds specifically to urokinase plasminogen activator receptor (uPAR).

In one aspect, the disclosure provides an engineered IL-12 p40 polypeptide,
30 comprising one or more amino acid substitutions at positions C177 and C252 of SEQ ID NO: 84.

In certain embodiments, the C177 amino acid substitution is selected from the group consisting of C177S, C177F, C177M, C177H, C177I, or C177Q.

In certain embodiments, the C252 amino acid substitution is C252S.

In certain embodiments, the p40 comprises an amino acid sequence of SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87, or an amino acid sequence comprising at least about 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence similarity or identity to the amino acid sequences as set forth in SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87.

In a further aspect, the disclosure provides a fusion protein comprising: (i) the engineered IL-12 p40 polypeptide recited above; and (ii) one or more fusion partners (e.g., a functional moiety or anti-uPAR antigen binding protein).

In certain embodiments, the one or more fusion partners comprise a moiety that reduces aggregation of the engineered IL-12 p40 polypeptide.

In certain embodiments, the one or more fusion partners comprise a moiety that increases expression of the engineered IL-12 p40 polypeptide.

In certain embodiments, the one or more fusion partners comprise a moiety that increases serum half-life of the engineered IL-12 p40 polypeptide.

In certain embodiments, the one or more fusion partners comprise a targeting moiety.

In certain embodiments, the one or more fusion partners comprise a serum albumin, polyethylene glycol (PEG), or an antigen binding protein.

In certain embodiments, the antigen binding protein binds specifically to urokinase plasminogen activator receptor (uPAR).

Exemplary IL-12 amino acid sequences are recited below in **Table 2**.

Table 2 – IL-12 Amino Acid Sequences

Sequence ID	Sequence
p35 (WT)	RNLPVATPDPGMFPC LHHSQNLLRAVS NMLQKARQTLEFYPC TSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGS CLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMDPKR QIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKL CILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 81)
p35 (C74K mutation)	RNLPVATPDPGMFPC LHHSQNLLRAVS NMLQKARQTLEFYPC TSEEIDHEDITKDKTSTVEACLPLELTKNESKLNRETSFITNGS CLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMDPKR

	QIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKL CILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 82)
p35 (S27D, C74K, and D188N mutations)	RNLPVATPDPGMFPCCLHHSQNLLRAVDNMLQKARQTLEFYPC TSEEIDHEDITKDKTSTVEACLPLELTKNESKLNRETSFITNGS CLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMDPKR QIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKL CILLHAFRIRAVTINRVMSYLNAS (SEQ ID NO: 83)
p40 (WT)	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSSHLLLLHK KEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVE CQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPD PPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSE WASVPCS (SEQ ID NO: 84)
p40 (C177S mutation)	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSSHLLLLHK KEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVE CQEDSASPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPD PKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSE WASVPCS (SEQ ID NO: 85)
p40 (C252S mutation)	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSSHLLLLHK KEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVE CQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPD PKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSE WASVPCS (SEQ ID NO: 86)
p40 (C177S / C252S mutation)	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSSHLLLLHK KEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVE CQEDSASPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPD PKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSE WASVPCS (SEQ ID NO: 87)
scIL-12 (WT p40 - WT p35)	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLD QSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSSHLLLLHK KEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVE CQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPD PPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSE

	WASVPCSGGSGGGGSGGRNLPVATPDPMFPC LHHSQNLLRA VSNMLQKARQTLEFYPC TSEEIDHEDITKDKTSTVEACLPLELT KNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIEDLKMY QVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSET VPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 88)
scIL-12 (WT p35 - WT p40)	RNLPVATPDPMFPC LHHSQNLLRAVSNMLQKARQTLEFYPC TSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGS CLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMDPKR QIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKL CILLHAFRIRAVTIDRVMSYLNASGGSGGGGSGGIWELKKDVY VVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSSEVLGSGK TLTIQVKEFGDAGQYTCHKGGEVLSHLLLLHKKEDGIWSTDI LKDQKEPKNKTFRLRCEAKNYSGRFTCWWTITSTDLTFSVKSS RGSSDPQGVTCGAATLSAERVGRDNKEYEYSVEQCEDSACPA AEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPL KNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDR VFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS (SEQ ID NO: 89)

Fusion Proteins

The urokinase plasminogen activator receptor (uPAR) binding polypeptides described
5 herein, including the uPAR antigen binding proteins described above, can be linked (i.e.,
attached or fused) to one or both of 1) a cytokine or variant thereof, and 2) a checkpoint
molecule modulator. The uPAR antigen binding proteins are useful for targeting the linked
protein (i.e., cytokine or checkpoint molecule modulator) to a uPAR-expressing tumor.

The phrase “checkpoint molecule modulator” refers to proteins or peptides that are
10 capable of agonizing or antagonizing checkpoint molecules as appropriate to enhance a
desirable immune response (e.g., agonizing immune stimulatory checkpoint molecules or
antagonizing immune inhibitory checkpoint molecules) or to inhibit an undesirable immune
response (e.g., antagonizing immune stimulatory checkpoint molecules for an inappropriate
response). Stimulatory or costimulatory checkpoint molecules include, but are not limited to:
15 CD27, CD70, CD28, CD40, CD40 ligand, CD122, CD137, OX40, GITR and its ligands,
ICOS, CD131 (4-1BBB and its ligands). Inhibitory checkpoint molecules include, but are not
limited to: Adenosine A2A receptor (A2AR), B7-H3, B7-H4, BTLA (B and T lymphocyte
attenuator), CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), IDO (indoleamine 2,3,

dioxygenase), KIR (killer-cell immunoglobulin-like receptor), LAG3 (lymphocyte activation gene-3), PD-1 (programmed cell death-1 receptor) and its ligands PD-L1 (programmed death-ligand 1) and PD-L2 (programmed death-ligand 2), TIM-3 (T-cell immunoglobulin and mucin domain-containing protein 3), VISTA (v-domain Ig suppressor of T cell activation).

- 5 Checkpoint molecule modulators used in the fusion proteins of the disclosure may include an inhibitory or stimulatory checkpoint molecule, a functional fragment thereof, a functional derivative thereof or a circularly permuted variant thereof.

In addition to antigen binding proteins, the uPAR binding polypeptide can comprise a peptide derived from integrins that block uPAR integration interactions. One such peptide is
10 M25 having the amino acid sequence PRYQHIGLVAMFRQNTG (SEQ ID NO: 157), a peptide derived from the β_2 subunit of Mac-1 inhibited leukocyte adhesion to fibrinogen, vitronectin, and cytokine-stimulated endothelial cells. M25 also blocked the association of uPAR with β_1 and impaired β_1 -integrin-dependent spreading and migration of human vascular smooth muscle cells on fibronectin and collagen (Simon DI, et al. (2000) *J Biol*
15 *Chem.* **275**:10228–34). A second peptide that inhibits uPAR-integrin complex formation, P25 having the amino acid sequence AESTYHHLSLGYMYTLN (SEQ ID NO: 158), was able to decrease tumor cell attachment to vitronectin and increase tumor cell attachment to fibronectin (van der Pluijm G, et al. (2001) *Am J Pathol.* **159**:971–82).

In one aspect, the disclosure provides a fusion protein comprising: (i) an antigen
20 binding protein that binds specifically to urokinase plasminogen activator receptor (uPAR); and (ii) a cytokine or variant thereof.

In certain embodiments, the antigen binding protein and the cytokine or variant thereof are linked with an amino acid linker.

In certain embodiments, the cytokine or variant thereof is selected from the group
25 consisting of IL-2, circularly permuted IL-2 (cpIL-2), IL-15, circularly permuted IL15 (cpIL-15), IL-6, circularly permuted IL-6 (cpIL-6), IL-10, or IL-12.

In certain embodiments, the cytokine or variant thereof comprises at least one interleukin-12 (IL-12) subunit (e.g., p35 and p40). In certain embodiments, the IL-12 subunit comprises one or both of p35 and p40. For example, but in no way limiting, the fusion
30 protein comprises: (i) an antigen binding protein that binds specifically to uPAR; and (ii) a

p35 polypeptide. Alternatively, the fusion protein comprises: (i) an antigen binding protein that binds specifically to uPAR; and (ii) a p40 polypeptide.

In certain embodiments, the p35 polypeptide comprises one or more amino acid substitutions at positions M12, S27, N28, Q35, F39, S44, C74, M111, V114, M119, M145, F150, L161, and D188 of SEQ ID NO: 81. In certain embodiments, the amino acid substitution comprises M12S; S27D; N28R; Q35E; F39D or F39H; S44K; C74K, C74D, C74W, C74Y, or C74Q; M111F or M111W; V114I; M119L; M145L; F150Y; L161D or L161Q; D188N; or a combination thereof. In certain embodiments, the amino acid substitution comprises N28R and Q35E. In certain embodiments, the amino acid substitution comprises S27D and D188N. In certain embodiments, the amino acid substitution comprises C74K. In certain embodiments, the amino acid substitution comprises S27D, C74K, and D188N. In certain embodiments, the p35 comprises an amino acid sequence of SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83, or an amino acid sequence comprising at least about 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence similarity or identity to the amino acid sequences as set forth in SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.

In certain embodiments, the p40 polypeptide comprises one or more amino acid substitution at positions C177 and C252 of SEQ ID NO: 84. In certain embodiments, the C177 amino acid substitution is selected from the group consisting of C177S, C177F, C177M, C177H, C177I, or C177Q. In certain embodiments, the C252 amino acid substitution is C252S. In certain embodiments, the p40 polypeptide comprises an amino acid sequence of SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87, or an amino acid sequence comprising at least about 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence similarity or identity to the amino acid sequences as set forth in SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87.

In certain embodiments, the cytokine or variant thereof comprises a single-chain IL-12 (scIL-12) comprising a p35 polypeptide and a p40 polypeptide attached by an amino acid linker. In certain embodiments, the amino acid linker comprises the sequence GGSGGGSGG (SEQ ID NO: 156). In certain embodiments, the scIL-12 comprises an amino acid sequence of SEQ ID NO: 88 or SEQ ID NO: 89, or an amino acid sequence comprising at least about 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, or 99%) sequence similarity or identity to the amino acid sequences as set forth in SEQ ID NO: 88 or SEQ ID NO: 89.

In certain embodiments, the antigen binding protein of the fusion protein comprises:

5 a) a VH domain comprising an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5); and

b) a VL domain comprising an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), and an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8).

10 In certain embodiments, the antigen binding protein of the fusion protein comprises:

a) a VH domain comprising an HCDR1 amino acid sequence of GFSLTNYG (SEQ ID NO: 11), an HCDR2 amino acid sequence of IWSDGGT (SEQ ID NO: 12), an HCDR3 amino acid sequence of ARGGRSDLFAY (SEQ ID NO: 13); and

15 b) a VL domain comprising an LCDR1 amino acid sequence of QSIVHSNGNTY (SEQ ID NO: 14), an LCDR2 amino acid sequence of KVS (SEQ ID NO: 15), and an LCDR3 amino acid sequence of FQGSHVPYT (SEQ ID NO: 16).

In certain embodiments, the antigen binding protein of the fusion protein comprises:

20 a) a VH domain comprising an HCDR1 amino acid sequence of GNTFTDYG (SEQ ID NO: 19), or GTTFTDYG (SEQ ID NO: 41), an HCDR2 amino acid sequence of INTNTGEP (SEQ ID NO: 20), an HCDR3 amino acid sequence of AHYSFDY (SEQ ID NO: 21); and

b) a VL domain comprising an LCDR1 amino acid sequence of ENIYSN (SEQ ID NO: 22), an LCDR2 amino acid sequence of AAT (SEQ ID NO: 23), and an LCDR3 amino acid sequence of QHFWGTPWT (SEQ ID NO: 24).

25 In certain embodiments, the antigen binding protein of the fusion protein comprises:

a) a VH domain comprising an HCDR1 amino acid sequence of GYTFTSYG (SEQ ID NO: 27), an HCDR2 amino acid sequence of IYPRSGNT (SEQ ID NO: 28), an HCDR3 amino acid sequence of AGKDYGSTYADY (SEQ ID NO: 29); and

30 b) a VL domain comprising an LCDR1 amino acid sequence of SSVSSRY (SEQ ID NO: 30), an LCDR2 amino acid sequence of GTS (SEQ ID NO: 31), and an LCDR3 amino acid sequence of QQYHSDPLT (SEQ ID NO: 32).

In certain embodiments, the antigen binding protein of the fusion protein comprises:

- a) a VH domain comprising an amino acid sequence of SEQ ID NO: 33, and a VL domain comprising an amino acid sequence of SEQ ID NO: 35;
- b) a VH domain comprising an amino acid sequence of SEQ ID NO: 33, and a VL domain comprising an amino acid sequence of SEQ ID NO: 36;
- c) a VH domain comprising an amino acid sequence of SEQ ID NO: 34, and a VL domain comprising an amino acid sequence of SEQ ID NO: 35;
- d) a VH domain comprising an amino acid sequence of SEQ ID NO: 34, and a VL domain comprising an amino acid sequence of SEQ ID NO: 36;
- e) a VH domain comprising an amino acid sequence of SEQ ID NO: 37, and a VL domain comprising an amino acid sequence of SEQ ID NO: 38;
- f) a VH domain comprising an amino acid sequence of SEQ ID NO: 37, and a VL domain comprising an amino acid sequence of SEQ ID NO: 39;
- g) a VH domain comprising an amino acid sequence of SEQ ID NO: 37, and a VL domain comprising an amino acid sequence of SEQ ID NO: 40;
- h) a VH domain comprising an amino acid sequence of SEQ ID NO: 42, and a VL domain comprising an amino acid sequence of SEQ ID NO: 44; or
- i) a VH domain comprising an amino acid sequence of SEQ ID NO: 43, and a VL domain comprising an amino acid sequence of SEQ ID NO: 44.

In certain embodiments, the VH domain and VL domain are attached with an amino acid linker. In certain embodiments, the amino acid linker comprises (GGGGS)_n (SEQ ID NO: 148), wherein n is an integer between 1 and 5. In certain embodiments, the amino acid linker comprises the amino acid sequence GGGSGGGSGGGGS (SEQ ID NO: 149) or GGS GGGSGGGSGGGSGGGSGGGSGG (SEQ ID NO: 150).

In certain embodiments, the antigen binding protein comprises a Fab fragment, a F(ab')₂ fragment, an Fd fragment, an Fv fragment, a single chain Fv (scFv), a dAb fragment, a single domain antibody, or a nanobody.

In certain embodiments, the antigen binding protein comprises the amino acid sequence of any one of SEQ ID NOs: 56-71.

In certain embodiments, the antigen binding protein further comprises the amino acid sequence of any one of SEQ ID NOs: 72-78.

In certain embodiments, the antigen binding protein comprises an antibody heavy chain (HC) amino acid sequence of SEQ ID NO: 45, 46, 49, 53, or 54, and an antibody light chain (LC) amino acid sequence of SEQ ID NO: 47, 48, 50, 51, 52, and 55.

In certain embodiments, the antigen binding protein comprises:

- 5 a) an antibody HC comprising the amino acid sequence of SEQ ID NO: 45, and an antibody LC comprising the amino acid sequence of SEQ ID NO: 47;
- b) an antibody HC comprising the amino acid sequence of SEQ ID NO: 45, and an antibody LC comprising the amino acid sequence of SEQ ID NO: 48;
- c) an antibody HC comprising the amino acid sequence of SEQ ID NO: 46, and an
10 antibody LC comprising the amino acid sequence of SEQ ID NO: 47;
- d) an antibody HC comprising the amino acid sequence of SEQ ID NO: 46, and an antibody LC comprising the amino acid sequence of SEQ ID NO: 48;
- e) an antibody HC comprising the amino acid sequence of SEQ ID NO: 49, and an antibody LC comprising the amino acid sequence of SEQ ID NO: 50;
- 15 f) an antibody HC comprising the amino acid sequence of SEQ ID NO: 49, and an antibody LC comprising the amino acid sequence of SEQ ID NO: 51;
- g) an antibody HC comprising the amino acid sequence of SEQ ID NO: 49, and an antibody LC comprising the amino acid sequence of SEQ ID NO: 52;
- h) an antibody HC comprising the amino acid sequence of SEQ ID NO: 53, and an
20 antibody LC comprising the amino acid sequence of SEQ ID NO: 55; or
- i) an antibody HC comprising the amino acid sequence of SEQ ID NO: 54, and an antibody LC comprising the amino acid sequence of SEQ ID NO: 55.

In another aspect, the disclosure provides a combination comprising a first fusion protein and a second fusion protein, wherein:

- 25 (a) the first fusion protein comprises:
 - (i) an antigen binding protein that binds specifically to a first epitope on urokinase plasminogen activator receptor (uPAR); and
 - (ii) a IL-12 p35 subunit or variant thereof; and
- (b) the second fusion protein comprises:
 - 30 (i) an antigen binding protein that binds specifically to a second epitope on uPAR; and
 - (ii) a IL-12 p40 subunit or variant thereof.

In certain embodiments, the first epitope and second epitope are the same.

In certain embodiments, the first epitope and second epitope are different.

5 Functional Moieties

The proteins of the disclosure (e.g., the uPAR binding proteins, the cytokines, and the fusion proteins) may be further conjugated (i.e., linked, attached, or fused) to functional moieties that impart desirable bioactivity on the proteins of the disclosure. Functional moieties include both protein functional moieties and non-protein functional moieties (e.g.,
10 PEG or polysaccharides). For example, but in no way limiting, a cytokine or variant thereof may be conjugated via an optional linker to the Fc region of an immunoglobulin or to human serum albumin (HSA) for the purpose of extending the half-life of the cytokine or variant thereof (e.g., an IL-12 p35-HSA or an IL-12 p40-HSA conjugate).

In certain embodiments, the functional moiety is an Fc region (i.e., Fc domain) of an
15 immunoglobulin. In certain embodiments, the Fc region can be linked to the proteins of the disclosure (e.g., uPAR binding proteins, cytokines, and fusion proteins) via a single chain linker as described in WO 2016/100788, incorporated herein by reference. These linkers comprise a constant region of an immunoglobulin light chain (CL) linked to a CH1 constant region of an immunoglobulin heavy chain (scCLCH1 or scCH1CL linkers) fused to an Fc
20 region of an immunoglobulin. For example, a fusion protein of the disclosure (e.g., a cytokine linked to a anti-uPAR antigen binding protein) may be further linked to an Fc region of an immunoglobulin via scCLCH1 or scCH1CL linkers. This type of linker confers favorable properties on the fusion protein of the disclosure of such as improved bioactivity and increased half-life on the fusion proteins of the disclosure.

25 The fusion proteins of the disclosure may be further stabilized *in vivo* and their half-life increased by binding to serum albumin molecules e.g. human serum albumin (HSA) which resist degradation and/or clearance or sequestration. These serum albumin molecules are naturally occurring proteins which themselves have a long half-life *in vivo*.

30

Linkers

Any of the proteins described herein (e.g., the uPAR binding proteins, the cytokine proteins, and the fusion proteins) can include one or more (e.g., two or more, three or more, four or more, five or more, six or more, or seven or more) linker moieties, which link one or more (or all) of the polypeptides of the fusion protein, link one or more cytokine proteins, or link one or more uPAR binding proteins (e.g., link the VH and VL domains of a uPAR antigen binding protein). In certain embodiments, the linker is an amino acid linker that can be one amino acid in length, but can be longer in length. For example, a linker peptide can be at least two (e.g., at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, or at least 55 or more) amino acids long. Linker peptides can contain any amino acids.

In certain embodiments, any one or more linkers present in the proteins of the disclosure are selected from artificial flexible polypeptides comprising amino acids selected from Gly (G), and/or Ser (S). In certain embodiments, the linker is comprised of polypeptide of the general formula (GGGS (SEQ ID NO: 151))_n or (GGGGS (SEQ ID NO: 152))_n or (SGGSGGG (SEQ ID NO: 153))_n or (GGSGGSG (SEQ ID NO: 154))_n wherein n is an integer from 1 to 10. In certain embodiments, each linker is a polypeptide comprising from about 1 to about 100 amino acids, such as about 1-50 amino acids, about 1-25 amino acids, about 1-15 amino acids, about 1-10 amino acids, about 4-24 amino acids, about 5-20 amino acids, about 5-15 amino acids, and about 5-10 amino acids. In certain embodiments, the linker is (GGGGS (SEQ ID NO: 155))_n wherein n is 2 or 4. Any linker may further comprise amino acids such as, for example, Lys (K), Thr (T), Glu (E), and Asp (D).

In certain embodiments, the amino acid linker comprises (GGGGS)_n (SEQ ID NO: 148), wherein n is an integer between 1 and 5. In certain embodiments, the amino acid linker comprises the amino acid sequence GGGSGGGSGGGGS (SEQ ID NO: 149) or GGSGGGSGGGSGGGSGGGSGGGSGG (SEQ ID NO: 150). In certain embodiments, the amino acid linker comprises the amino acid sequence GGS.

In certain embodiments, the linker may comprise one or more mucin proteins or mucin domains of proteins (e.g., any protein encoded for by a MUC gene (e.g., MUC1, MUC2,

MUC3A, MUC3B, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC9, MUC11, MUC12, MUC13, MUC15, MUC16, MUC17, MUC19, MUC20, MUC21). Mucin domain proteins and polypeptides contain a high degree of glycosylation which structurally allows mucin proteins and other polypeptides comprising mucin domains to behave as stiffened random coils. The rod-like nature of the mucin domains can rigidly separate the bioactive protein (e.g., the cytokine) from the fusion partner (e.g., uPAR binding protein), and thereby be less susceptible to loss in activity either fusion partner.

Such mucin domain polypeptides useful in accordance with the disclosure are described in WO 2013/184939 and WO 2013/184938, incorporated herein by reference. These linkers are useful to provide optimal spacing between the polypeptides of the fusion proteins of the disclosure (e.g., between the ATF polypeptide and cytokine polypeptide) or for example, to provide an increase in half-life of the fusion protein as a whole regardless of location of the mucin domain in the fusion protein. For example, a mucin-domain may be present at the N-terminus or C-terminus of the fusion protein. Mucin domain polypeptide linkers may further be linked to the Fc region of an immunoglobulin polypeptide that may also function to increase half-life of the fusion protein of the invention as is described in WO 2013/184938.

Purification Tags

Any of the proteins described herein (e.g., the uPAR binding proteins, the cytokine proteins, and the fusion proteins) can include one or more (e.g., two or more, three or more, four or more, five or more, six or more, or seven or more) purification tags, which facilitate the purification of the proteins described herein. In certain embodiments, the purification tag is an avi tag (GLNDIFEAQKIEWHE; SEQ ID NO: 240). In certain embodiments, the purification tag is a 6xHis tag (HHHHHH; SEQ ID NO: 241). One or both of the avi tag and 6xHis tag may be present on a single protein and in any order. In certain embodiments, the purification tag is linked to a protein described herein with a gly-ser linker. In certain embodiments, the gly-ser linker comprises GGS and/or GGSGGG (SEQ ID NO: 242). In certain embodiments, the purification is any one of GGSHHHHHHGGSGGLNDIFEAQKIEWHE (SEQ ID NO: 243),

GGSGGHHHHHHGGSGGLNDIFEAQKIEWHE (SEQ ID NO: 244), and
GGSGGLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 245).

Expression of Antigen Binding Proteins, Cytokines, and Fusion Proteins

5 In one aspect, polynucleotides or nucleic acids encoding the antigen binding proteins (e.g., anti-uPAR antigen binding proteins), cytokines or variants thereof (e.g., engineered IL-12 p35 or engineered IL-12 p40), or fusion proteins (e.g., anti-uPAR antigen binding protein – cytokine fusion proteins) disclosed herein are provided. Methods of making an antigen binding protein, cytokine, or fusion protein comprising expressing these polynucleotides are
10 also provided.

Polynucleotides encoding the antigen binding proteins, cytokines, or fusion proteins disclosed herein are typically inserted in an expression vector for introduction into host cells that may be used to produce the desired quantity of the antigen binding proteins or fusion proteins. Accordingly, in certain aspects, the disclosure provides expression vectors
15 comprising polynucleotides disclosed herein and host cells comprising these vectors and polynucleotides.

The term “vector” or “expression vector” is used herein to mean vectors used in accordance with the present invention as a vehicle for introducing into and expressing a desired gene in a cell. As known to those skilled in the art, such vectors may readily be
20 selected from the group consisting of plasmids, phages, viruses and retroviruses. In general, vectors compatible with the instant invention will comprise a selection marker, appropriate restriction sites to facilitate cloning of the desired gene and the ability to enter and/or replicate in eukaryotic or prokaryotic cells.

Numerous expression vector systems may be employed for the purposes of this
25 invention. For example, one class of vector utilizes DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (e.g., RSV, MMTV, MOMLV or the like), or SV40 virus. Others involve the use of polycistronic systems with internal ribosome binding sites. Additionally, cells which have integrated the DNA into their chromosomes may be selected by introducing
30 one or more markers which allow selection of transfected host cells. The marker may provide

for prototrophy to an auxotrophic host, biocide resistance (e.g., antibiotics) or resistance to heavy metals such as copper. The selectable marker gene can either be directly linked to the DNA sequences to be expressed, or introduced into the same cell by co-transformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may
5 include signal sequences, splice signals, as well as transcriptional promoters, enhancers, and termination signals. In some embodiments, the cloned variable region genes are inserted into an expression vector along with the heavy and light chain constant region genes (e.g., human constant region genes) synthesized as discussed above.

In other embodiments, the antigen binding proteins may be expressed using
10 polycistronic constructs. In such expression systems, multiple gene products of interest such as heavy and light chains of antibodies may be produced from a single polycistronic construct. These systems advantageously use an internal ribosome entry site (IRES) to provide relatively high levels of polypeptides in eukaryotic host cells. Compatible IRES sequences are disclosed in U.S. Pat. No. 6,193,980, which is incorporated by reference herein in its entirety for all
15 purposes. Those skilled in the art will appreciate that such expression systems may be used to effectively produce the full range of polypeptides disclosed in the instant application.

More generally, once a vector or DNA sequence encoding an antibody, or fragment thereof, has been prepared, the expression vector may be introduced into an appropriate host cell. That is, the host cells may be transformed. Introduction of the plasmid into the host cell
20 can be accomplished by various techniques well known to those of skill in the art. These include, but are not limited to, transfection (including electrophoresis and electroporation), protoplast fusion, calcium phosphate precipitation, cell fusion with enveloped DNA, microinjection, and infection with intact virus. See, Ridgway, A. A. G. "Mammalian Expression Vectors" Chapter 24.2, pp. 470-472 Vectors, Rodriguez and Denhardt, Eds.
25 (Butterworths, Boston, Mass. 1988). Plasmid introduction into the host can be by electroporation. The transformed cells are grown under conditions appropriate to the production of the light chains and heavy chains, and assayed for heavy and/or light chain protein synthesis. Exemplary assay techniques include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), fluorescence-activated cell sorter analysis (FACS),
30 immunohistochemistry and the like.

As used herein, the term “transformation” shall be used in a broad sense to refer to the introduction of DNA into a recipient host cell that changes the genotype and consequently results in a change in the recipient cell.

Along those same lines, “host cells” refers to cells that have been transformed with
5 vectors constructed using recombinant DNA techniques and encoding at least one heterologous gene. In descriptions of processes for isolation of polypeptides from recombinant hosts, the terms “cell” and “cell culture” are used interchangeably to denote the source of antibody unless it is clearly specified otherwise. In other words, recovery of polypeptide from the “cells” may mean either from spun down whole cells, or from the cell
10 culture containing both the medium and the suspended cells.

In one embodiment, a host cell line used for antibody expression or fusion protein expression is of mammalian origin. Those skilled in the art can determine particular host cell lines which are best suited for the desired gene product to be expressed therein. Exemplary host cell lines include, but are not limited to, DG44 and DUXB11 (Chinese hamster ovary
15 lines, DHFR minus), HELA (human cervical carcinoma), CV-1 (monkey kidney line), COS (a derivative of CV-1 with SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), HAK (hamster kidney line), SP2/O (mouse myeloma), BFA-1c1BPT (bovine endothelial cells), RAJI (human lymphocyte), 293 (human kidney) and the like. In one embodiment, the cell line provides for altered glycosylation, e.g., afucosylation, of the
20 antibody expressed therefrom (e.g., PER.C6® (Crucell) or FUT8-knock-out CHO cell lines (Potelligent® cells) (Biowa, Princeton, N.J.)). Host cell lines are typically available from commercial services, e.g., the American Tissue Culture Collection, or from published literature.

In vitro production allows scale-up to give large amounts of the desired polypeptides.
25 Techniques for mammalian cell cultivation under tissue culture conditions are known in the art and include homogeneous suspension culture, e.g., in an airlift reactor or in a continuous stirrer reactor, or immobilized or entrapped cell culture, e.g., in hollow fibers, microcapsules, on agarose microbeads or ceramic cartridges. If necessary and/or desired, the solutions of polypeptides can be purified by the customary chromatography methods, for example gel

filtration, ion-exchange chromatography, chromatography over DEAE-cellulose and/or (immuno-) affinity chromatography.

Genes encoding the antigen binding proteins featured in the invention can also be expressed non-mammalian cells such as bacteria or yeast or plant cells. In this regard it will be appreciated that various unicellular non-mammalian microorganisms such as bacteria can also be transformed, i.e., those capable of being grown in cultures or fermentation. Bacteria, which are susceptible to transformation, include members of the enterobacteriaceae, such as strains of *Escherichia coli* or *Salmonella*; *Bacillaceae*, such as *Bacillus subtilis*; *Pneumococcus*; *Streptococcus*, and *Haemophilus influenzae*. It will further be appreciated that, when expressed in bacteria, the proteins can become part of inclusion bodies. The proteins must be isolated, purified and then assembled into functional molecules.

In addition to prokaryotes, eukaryotic microbes may also be used. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among eukaryotic microorganisms, although a number of other strains are commonly available. For expression in *Saccharomyces*, the plasmid YRp7, for example (Stinchcomb et al., Nature, 282:39 (1979); Kingsman et al., Gene, 7:141 (1979); Tschemper et al., Gene, 10:157 (1980)), is commonly used. This plasmid already contains the TRP1 gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, Genetics, 85:12 (1977)). The presence of the trp1 lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Methods of Administering Antigen Binding Proteins, Cytokines, and Fusion Proteins

Methods of preparing and administering antigen binding proteins, cytokines, or fusion proteins of the disclosure as well as the nucleic acids described herein, the vectors described herein, the host cell cells described herein or the compositions described herein to a subject are well known to or are readily determined by those skilled in the art. The route of administration of the antigen binding proteins of the current disclosure may, e.g., be oral, parenteral, by inhalation, or topical. The term parenteral as used herein includes intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, rectal or vaginal administration. In

certain embodiments, the antigen binding proteins or fusion proteins are administered intravenously. The term intraocular as used herein includes, but is not limited to, subconjunctival, intravitreal, retrobulbar, or intracameral. The term topical as used herein includes, but is not limited to, administration with liquid or solution eye drops, emulsions
5 (e.g., oil-in-water emulsions), suspensions, and ointments.

While all these forms of administration are clearly contemplated as being within the scope of the current disclosure, a form for administration would be a solution for injection. Usually, a suitable pharmaceutical composition for injection may comprise a buffer (e.g., acetate, phosphate or citrate buffer), a surfactant (e.g., polysorbate), optionally a stabilizer
10 agent (e.g., human albumin), etc. However, in other methods compatible with the teachings herein, the modified antibodies can be delivered directly to the site of the adverse cellular population thereby increasing the exposure of the diseased tissue to the therapeutic agent.

Effective doses of the compositions of the present disclosure, for the treatment of the related conditions vary depending upon many different factors, including means of
15 administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human, but non-human mammals, including transgenic mammals, can also be treated. Treatment dosages may be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

20 As previously discussed, the antigen binding proteins or fusion proteins of the present disclosure, conjugates or recombinants thereof may be administered in a pharmaceutically effective amount for the in vivo treatment of mammalian disorders. In this regard, it will be appreciated that the disclosed antigen binding proteins will be formulated to facilitate administration and promote stability of the active agent.

25 Pharmaceutical compositions in accordance with the present disclosure typically include a pharmaceutically acceptable, non-toxic, sterile carrier such as physiological saline, nontoxic buffers, preservatives and the like. For the purposes of the instant application, a pharmaceutically effective amount of the antigen binding proteins shall be held to mean an amount sufficient to achieve effective binding to an antigen and to achieve a benefit, e.g., to
30 ameliorate symptoms of a disease or disorder or to detect a substance or a cell. In the case of

tumor cells, the antigen binding proteins will typically be capable of interacting with selected immunoreactive antigens on neoplastic or immunoreactive cells and provide for an increase in the death of those cells. Of course, the pharmaceutical compositions of the present disclosure may be administered in single or multiple doses to provide for a pharmaceutically effective
5 amount of the modified binding polypeptide.

In keeping with the scope of the present disclosure, the antigen binding proteins of the disclosure may be administered to a human or other animal in accordance with the aforementioned methods of treatment in an amount sufficient to produce a therapeutic or prophylactic effect. The antigen binding proteins of the disclosure can be administered to such
10 human or other animal in a conventional dosage form prepared by combining the antigen binding proteins of the disclosure with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and
15 other well-known variables. Those skilled in the art will further appreciate that a cocktail comprising one or more species of antigen binding proteins described in the current disclosure may prove to be particularly effective. Similarly, the nucleic acids described herein, the vectors described herein, the host cell cells described herein (in particular the immune cells bearing a CAR) or the compositions described herein may be administered to a human or
20 other animal in accordance with the methods of treatment described above in an amount sufficient to produce a therapeutic or prophylactic effect.

“Efficacy” or “in vivo efficacy” as used herein refers to the response to a therapy by the pharmaceutical composition of the disclosure, using e.g., standardized response criteria, such as standard ophthalmological response criteria. The success or in vivo efficacy of the
25 therapy using a pharmaceutical composition of the disclosure refers to the effectiveness of the composition for its intended purpose, i.e., the ability of the composition to cause its desired effect. The in vivo efficacy may be monitored by established standard methods for the specific diseases. In addition, various disease specific clinical chemistry parameters and other established standard methods may be used.

In some embodiments, the compounds and cells described herein are administered in combination with one or more different pharmaceutical compounds. Generally, therapeutic use of the compounds and cells described herein may be in combination with one or more therapies selected from the group of antibody therapy, chemotherapy, cytokine therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy, radiation therapy or vaccine therapy.

Methods of Treatment

Methods of treating diseases or disorders associated with uPAR expression in a patient by administering the antigen binding proteins, cytokines, or fusion proteins of the disclosure are described herein. In certain embodiments, the disease or disorder associated with uPAR expression is cancer (e.g., a uPAR-expressing cancer or uPAR-expressing tumor).

In an aspect, the disclosure provides a method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of the pharmaceutical compositions, uPAR binding proteins, or fusion proteins described herein.

In certain embodiments, the pharmaceutical composition or fusion protein is administered simultaneously with a cytokine or variant thereof or a second fusion protein, wherein the second fusion protein comprises a different cytokine or variant thereof from the first fusion protein.

In certain embodiments, the pharmaceutical composition or fusion protein is administered sequentially a cytokine or variant thereof or a second fusion protein, wherein the second fusion protein comprises a different cytokine or variant thereof from the first fusion protein.

In certain embodiments, the cytokine or variant thereof comprises a IL-12 p35 subunit or an IL-12 p40 subunit.

In certain embodiments, the fusion protein clearance rate in a tumor of the patient is slower than the fusion protein clearance rate in serum of the patient.

In one aspect, the disclosure provides a method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a fusion protein and a non-fusion IL-12 subunit, wherein:

(a) the fusion protein comprises:

- 5 (i) an antigen binding protein that binds specifically to urokinase plasminogen activator receptor (uPAR); and
 - (ii) a IL-12 p35 subunit or variant thereof, or a IL-12 p40 subunit or variant thereof; and
 - (b) the non-fusion IL-12 subunit comprising a IL-12 p35 subunit or variant thereof, or a IL-12 p40 subunit or variant thereof,
- 10 wherein when the fusion protein comprises a IL-12 p35 subunit or variant thereof, the non-fusion IL-12 subunit comprises a IL-12 p40 subunit or variant thereof, and when the fusion protein comprises a IL-12 p40 subunit or variant thereof, the non-fusion IL-12 subunit comprises a IL-12 p35 subunit or variant thereof.

In certain embodiments, the fusion protein and non-fusion IL-12 subunit are
15 administered sequentially.

In certain embodiments, one or both of the fusion protein and non-fusion IL-12 subunit are administered intravenously.

In certain embodiments, the fusion protein is administered first, followed by administering the non-fusion IL-12 subunit after a sufficient amount of time has elapsed for
20 the fusion protein to accumulate in a tumor of the patient.

In certain embodiments, the fusion protein is administered first, followed by administering the non-fusion IL-12 subunit after a sufficient amount of time has elapsed for the fusion protein concentration to substantially reduce in serum of the patient.

In one aspect, the disclosure provides a method of treating cancer in a patient in need
25 thereof comprising administering to the patient a therapeutically effective amount of a first fusion protein and a second fusion protein, wherein:

(a) the first fusion protein comprises:

- (i) an antigen binding protein that binds specifically to a first epitope on urokinase plasminogen activator receptor (uPAR); and
- 30 (ii) a IL-12 p35 subunit or variant thereof; and

(b) the second fusion protein comprises:

(i) an antigen binding protein that binds specifically to a second epitope on uPAR; and

(ii) a IL-12 p40 subunit or variant thereof.

In certain embodiments, the first epitope and second epitope are the same.

5 In certain embodiments, the first epitope and second epitope are different.

In certain embodiments, the first fusion protein and second fusion protein are administered sequentially.

In certain embodiments, one or both of the first fusion protein and second fusion protein are administered intravenously.

10 In certain embodiments, the first fusion protein is administered first, followed by administering the second fusion protein after a sufficient amount of time has elapsed for the first fusion protein to accumulate in a tumor of the patient.

In certain embodiments, the first fusion protein is administered first, followed by administering the second fusion protein after a sufficient amount of time has elapsed for the
15 first fusion protein concentration to substantially reduce in serum of the patient.

In certain embodiments, the second fusion protein is administered first, followed by administering the first fusion protein after a sufficient amount of time has elapsed for the second fusion protein to accumulate in a tumor of the patient.

In certain embodiments, the second fusion protein is administered first, followed by
20 administering the first fusion protein after a sufficient amount of time has elapsed for the second fusion protein concentration to substantially reduce in serum of the patient.

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods described herein may be made using suitable equivalents
25 without departing from the scope of the embodiments disclosed herein. Having now described certain embodiments in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting.

30

EXAMPLES

Example 1 – Generation & Characterization of Parental Anti-uPAR Antibodies

NZBW mice were immunized with alternating injection of recombinant human uPAR and mouse uPAR extracellular domain protein. Hybridomas were generated and screened
 5 against human and mouse uPAR in an ELISA and in uPAR overexpressing HEK293 cells to identify antibodies with binding specificity to human and mouse uPAR. Top clones were further screened by ELISA for binding to uPAR ligand uPA-bound variants, including 1) human uPAR with and without human uPA, 2) mouse uPAR with and without mouse uPA, and 3) human uPAR with an H47C/N259C mutation (a human uPAR variant mimicking
 10 ligand-bound conformation). The following amino acid sequences were used for screening:

Human uPAR-His-AviTag fusion

LRCMQCKTNGDCRVEECALGQDLCRTTIVRLWEEGEELELVEKSCTHSEKTNRTLSY
 RTGLKITSLETVVCGLDLCNQNSGRAVITYSRSRYLECISCGSSDMSCERGRHQSLQC
 15 RSPEEQCLDVVTHWIEGEEGRPKDDRHLRGCGYLPGCPGSNGFHNNDTFHFLKCCN
 TTKCNEGPILLELNPQNGRQCYSCKGNSTHGCSSEETFLIDCRGPMNQCLVATGT
 PKNQSYMVRGCATASMCQHAHLGDAFSMNHIDVSCCTKSGCNHPDLVQYRGGSH
 HHHHHGGSGLNDIFEAQKIEWHE

Mouse uPAR-His-AviTag fusion

LQCMQCESNQSCLVEECALGQDLCRTTVLREWQDDRELEVTVTRGCAHSEKTNRTMS
 YRMGSMIISLTETVCATNLCNRPRPGARGRAFPQGRYLECASCSTLDQSCERGREQSL
 QCRYPTHEHCIEVVTLQSTERSLKDEDYTRGCGSLPGCPGTAGFHSNQTFHFLKCCNYT
 20 HCNGGPVLDLQSFPPNGFQCYSCEGNNTLGCSEESLINCGRGPMNQCLVATGLDVL
 GNRSYTVRGCATASWCQGS HVADSFPTHLNVS VSCCHGSGCNSPTGGSHHHHHHGG
 SGLNDIFEAQKIEWHE

Human uPA-His-AviTag fusion

MYRMQLLSICIALSLALVTNSSNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPKKF
 30 GGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQTYHAHRSDAL
 QLGLGKHNYCRNPDNRRRPWCYVQVGLKPLVQECMVHDCAGGSHHHHHHGGSGL
 NDIFEAQKIEWHE

Mouse uPA-His-AviTag fusion

MYRMQLLSICIALSLALVTNSGSVLGAPDESNCGCQNGGVCVSYKYFSRIRRCSCPRK
 35 FQGEHCEIDASKTCYHGNGDSYRGKANTDTKGRPCLAWNAPAVLQKPYNHRPDAI
 SLGLGKHNYCRNPDNQKRPWCYVQIGLRQFVQECMVHDCGGSHHHHHHGGSGLND
 IFEAQKIEWHE

Antibodies designated M1, M5, M8, and M43 each bound human uPAR independent of the ligand uPA and were selected for further characterization.

The heavy chain variable domain (VH) and light chain variable domain (VL) amino acid sequences and HCDR1-3 and LCDR1-3 amino acid sequences for M1, M5, M8, and M43 are recited below in **Table 3**.

Table 3 – Parental Antibody Sequences

Sequence ID	Sequence
M1 VH	EVQLQQSGAELVRPGASVKLSCTASGFNIKDEYINWVKQRPEQGL EWIGWIDPENGDT EYASKFQGKATITADTSSNTAYLQLSSLTSED TAVYYCTGGNYVGWFPYWGQGTLVTVSA (SEQ ID NO: 1)
M1 VL	QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKR WLYDTSKLAGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQW SSNPPYTFGGGTKLEIK (SEQ ID NO: 2)
M1 HCDR1	GFNIKDEY (SEQ ID NO: 3)
M1 HCDR2	IDPENGDT (SEQ ID NO: 4)
M1 HCDR3	TGGNYVGWFPY (SEQ ID NO: 5)
M1 LCDR1	SSVSY (SEQ ID NO: 6)
M1 LCDR2	DTS (SEQ ID NO: 7)
M1 LCDR3	QQWSSNPPY (SEQ ID NO: 8)
M5 VH	QVQLKESGPGLVAPSQSLITCTVSGFSLTNYGVHWVRQPPGKDL EWLVVIWSDGGTTYNSALKSRLSISKDNSKSQVFLKMNSLQTDDT AMYYCARGGRSDFAYWGQGTLVTVSA (SEQ ID NO: 9)
M5 VL	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPG QSPKLLIYKVSNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDLGVYY CFQGSHVPYTFGGGTKLEIK (SEQ ID NO: 10)
M5 HCDR1	GFSLTNYG (SEQ ID NO: 11)
M5 HCDR2	IWSDGGT (SEQ ID NO: 12)
M5 HCDR3	ARGGRSDFAY (SEQ ID NO: 13)
M5 LCDR1	QSIVHSNGNTY (SEQ ID NO: 14)
M5 LCDR2	KVS (SEQ ID NO: 15)
M5 LCDR3	FQGSHVPYT (SEQ ID NO: 16)
M8 VH	QIQLVQSGPELKKPGETVKISCKASGNTFTDYGMNWVKQSPGKG LKWMGWINTNTGEPTYADDFKGRFAFSLETSASTAYLQINNLLKNE DMATYFCAHYSFDYWGGGTTTLTVSS (SEQ ID NO: 17)

M8 VL	DIQMTQSPASLSVSVGQTVTITCRTSENIYSNLAWYQQKPGKSPQL LVYAATNLADGVPSRFSGSGSGTQYSLKINSLQSEDFGSYYCQHF WGTPWTFGGGKLEIK (SEQ ID NO: 18)
M8 HCDR1	GNTFTDYG (SEQ ID NO: 19)
M8 HCDR2	INTNTGEP (SEQ ID NO: 20)
M8 HCDR3	AHYSFDY (SEQ ID NO: 21)
M8 LCDR1	ENIYSN (SEQ ID NO: 22)
M8 LCDR2	AAT (SEQ ID NO: 23)
M8 LCDR3	QHFWGTPWT (SEQ ID NO: 24)
M43 VH	QVQLQQSGAELARPGASVNLSCASGYTFTSYGISWVKQRTGQG LEWIGEIYPRSGNTYYNEKFKGKATLTADKSSRTAYMELRSLTSE DSAVYFCAGKDYGSTYADYWQGQTTLVSS (SEQ ID NO: 25)
M43 VL	QIVLTQSPAISASPGEKVTMTCSASSSVSSRYLHWYQQKSGASP KLWIYGTSNLAGVPARFSGSGSGTSYSLTINSVEAEDAATYYCQ QYHSDPLTFGAGTKLELK (SEQ ID NO: 26)
M43 HCDR1	GYTFTSYG (SEQ ID NO: 27)
M43 HCDR2	IYPRSGNT (SEQ ID NO: 28)
M43 HCDR3	AGKDYGSTYADY (SEQ ID NO: 29)
M43 LCDR1	SSVSSRY (SEQ ID NO: 30)
M43 LCDR2	GTS (SEQ ID NO: 31)
M43 LCDR3	QQYHSDPLT (SEQ ID NO: 32)

The binding affinity of anti-uPAR antibodies M1, M5, M8, and M43 was determined. Binding affinity analysis was carried out using the Octet platform based on bio-layer interferometry (BLI) technology. Briefly, uPAR antibodies were immobilized via an Anti-
5 mouse Fc (AMC) sensor. Each uPAR antibody was tested at a concentration of 10 µg/mL. The load was histidine-tagged human uPAR (Sino#10925-H08H). A bare sensor was used as a control. The binding affinities of each antibody against human uPAR are recited below in **Table 4**.

10 Table 4 – Parental Antibody Binding Affinity Data

uPAR Antibody	KD (M)	k on(1/Ms)	k dis(1/s)	Full R ²
M8	1.08E-09	5.84E+05	6.30E-04	0.994079

M43	7.47E-09	1.18E+06	8.83E-03	0.998659
M1	5.11E-10	6.64E+05	3.40E-04	0.999416
M5	3.31E-08	2.91E+04	9.62E-04	0.983345

An additional binding affinity analysis was carried out using the Octet platform with histidine-tagged human uPAR immobilized on a sensor at a concentration of 10 µg/mL. The load was each of M1, M5, M8, and M43. A bare sensor was used as a control. The binding affinities of each antibody against human uPAR when human uPAR was immobilized are recited below in **Table 5**. Each antibody showed less than 1 pM binding affinity, suggesting avidity effects.

Table 5 – Binding Affinity Data

uPAR Antibody	KD (M)	k on(1/Ms)	k dis(1/s)	Full R ²
M8	<1.0E-12	3.11E+05	<1.0E-07	0.985572
M43	<1.0E-12	5.54E+05	<1.0E-07	0.990357
M1	<1.0E-12	4.48E+05	<1.0E-07	0.982889
M5	<1.0E-12	2.83E+04	<1.0E-07	0.985581

10

The uPAR binding epitopes for each antibody were characterized using the Octet platform recited above. Biotinylated human uPAR variant, uPAR DII-DIII domain or uPAR DIII domain, were captured by an SA sensor at 10 µg/ml for 180 seconds, followed by binding of M1, M5, M8, M43 or ATN 568 for 150 seconds at a concentration of 200 nM. The following amino acid sequences were used for the DII-DIII domain and DIII domain:

15

Human uPAR DII-DIII domain HisAvi Tag

MYRMQLLSIALSLALVTNSGNSGRAVTYSRSRYLECISCGSSDMSCERGRHQS LQC
 RSPEEQCLDVVTHWIQEGEEGRPKDDRHLRGCGYLPGCPGSNGFHNNDTFHFLKCCN
 TTKCNEGPILLENLPQNGRQCYSCKGNSTHGCSSEETFLIDCRGPMNQCLVATGTHE

20

PKNQSYMVRGCATASMCQHAHLGDAFSMNHIDVSCCTKSGCNHPDLDVQYRGGSH
HHHHHGGGSLNDIFEAQKIEWHE

Human uPAR DIII domain HisAvi Tag

5 MYRMQLLSICIALSLALVTNSGPILELENLPQNGRQCYSCKGNSTHGCSSSETFLIDCR
GPMNQCLVATGTHEPKNQSYMVRGCATASMCQHAHLGDAFSMNHIDVSCCTKSGC
NHPDLDVQYRGGSHHHHHHGGGSLNDIFEAQKIEWHE

M1, M5, M8, and M43 bind to the DII-DIII domain of human uPAR, while M1 is
10 capable of binding DIII alone as well (**FIG. 1A, FIG. 1B**). Each of M1, M5, M8, and M43
were found to bind to uPAR independent of uPA binding to uPAR, the uPAR ligand. The
anti-uPAR antibody ATN-658 was used as a comparator.

To test antibody and uPA ligand competition binding, human uPA was captured by an
SA sensor at 3.2 µg/ml (200 nM) for 45 seconds. Human recombinant protein uPAR-His was
15 loaded at 100 nM for 180 seconds, followed by M1, M5, M8, M43 or ATN 568 for 150
seconds at a concentration of 200 nM.

An antibody competition assay was performed using the Octet platform. The antibody
competition assay was performed by capturing biotinylated human uPAR on SA sensors. M1,
M5, M8, M43 and ATN-658 were then loaded as the first ligand to bind human uPAR at a
20 predetermined saturation concentration. Once saturation was reached by the first ligand, M1,
M5, M8, M43, and ATN-568 was then loaded as the second ligand to compete against M1,
M5, M8, M43 or ATN-658. The second ligand binding solution contained the same
concentration of the first preload ligand to prevent its disassociation during second ligand
binding. M1 was found to only compete with the comparator antibody, ATN-658. M5
25 competed against M43. None of M1, M8, or M43 competed for binding against each other to
human uPAR, indicating that the three antibodies have unique epitopes to human uPAR (i.e.,
the antibodies are non-competitive). The matrix competition assay data is recited below in
Table 6.

30 Table 6 – Antibody Competition Summary Data. Values demonstrating competition are in
bold.

Antibody	M1	M5	M8	M43	ATN-368	Buffer
M1	-0.0441	0.1611	0.3019	0.3122	-0.0144	-0.0137
M5	0.3646	-0.0173	0.3211	0.0175	0.3865	-0.0149
ATN-368	-0.0232	0.1793	0.3104	0.3421	-0.0274	-0.0053
None	0.3049	0.1809	0.2841	0.295	0.3304	-0.0009

The antibodies were next tested for their ability to bind human and mouse uPAR expressed on the surface of cells. Human HEK293 cells engineered to express human uPAR or mouse uPAR were dye-labeled and mixed with unlabeled parental HEK293 cells. Cells were incubated with 100 nM each of human and mouse uPA (uPAR ligand) for 30 min and then treated with a titration of the uPAR antibodies for 30 min. uPAR antibody binding was detected using anti-mouse-IgG-phycoerythrin (PE)-labelled secondary antibody. Median fluorescent intensity (MFI) values across the tested antibody concentrations are shown in **FIG. 2A** and **FIG. 2B**. The EC₅₀ values are recited below in **Table 7**. EC₅₀ values of cell binding without the uPA ligand were similar to experiments with the uPA ligand. M1, M5, M8, M43 EC₅₀ binding values were all superior to the comparator ATN-658.

Table 7 – EC₅₀ values for anti-PAR antibodies in the cell-binding assay

Antibodies	EC ₅₀ (nM)	
	Hu-uPAR	Ms-uPAR
M43	0.30	2.83
M8	0.54	31.35
M5	2.53	93.81
M1	0.66	-
ATN-658	3.99	-

Example 2 – Humanization of Parental Anti-uPAR Antibodies

Anti-uPAR parental antibodies M1, M8, and M43 described above were carried forward for humanization. Various parameters for each antibody were first determined, including 1) the natural humanization percent identity of each mouse antibody to a human antibody sequence; 2) regions of immunogenicity (e.g., T-cell, B-cell, and MHC-II

antigenicity epitopes); 3) antibody misfolding issues (e.g., aggregation and thermal stability); and 4) post-translational modifications (PTMs). The PTMs included deamidation sites (e.g., Asn-Gly (NG), causing an asparagine to aspartate/isoaspartate change), isomerization sites (e.g., Asp-Gly (DG), Asp/Asp (DD), causing an aspartate to isoaspartate change), glycosylation sites (e.g., Asn-X-Ser/Thr (NXS) amino acid motifs, where “X” is any amino acid, such as NLS), oxidation sites (e.g., Met and/or Trp amino acids, which may be surface exposed and/or in a CDR of the antibody), proteolysis sites (e.g., Asp-Ser (DS) and/or Asp-Pro (DP) amino acid motifs).

Humanized antibodies were then generated. The heavy chain variable domain (VH) and light chain variable domain (VL) amino acid sequences and HCDR1-3 and LCDR1-3 amino acid sequences are recited below in **Table 8**. The full-length heavy chain (HC) and light chain (LC) amino acid sequences, which include the constant region of the antibody, are recited below in **Table 9**. Single chain variable (scFv) format antibody amino acid sequences are recited below in **Table 10**.

Table 8 – Humanized Antibody VH / VL & HCDR1-3 / LCDR1-3 Amino Acid Sequences

Sequence ID	Sequence
M1-H1 VH	EVQLVQSGAEVKKPGATVKISCTASGFNIKDEYINWVKQRPGKGL EWIGWIDPENGDT EYASKFQGRVTITADTSTD TAYLELSSLRSED AVYYCTGGNYVGWFPYWGQGT LTVVSS (SEQ ID NO: 33)
M1-H3 VH	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYINWVKQRPGQG LEWIGWIDPENGDT EYASKFQGRATITADTSTD TAYLELSSLRSED TAVYYCTGGNYVGWFPYWGQGT LTVVSS (SEQ ID NO: 34)
M1-L1 VL	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWYQQKPGTSPRRW IYDTSKSLASGVPARFSGSGSGTDFTLT ISSLEPEDFAVYYCQWSS NPPYTFGQGTKLEIK (SEQ ID NO: 35)
M1-L4 VL	QIVLTQSPATLSASPGERVMTSCSASSSVSYMHWYQQKPGTSPRR WLYDTSKSLASGVPARFSGSGSGTDYTLT ISSLEPEDFATYYCQW SSNPPYTFGQGTKLEIK (SEQ ID NO: 36)
M1-H1 & M1-H3 HCDR1	GFNIKDEY (SEQ ID NO: 3)
M1-H1 & M1-H3 HCDR2	IDPENGDT (SEQ ID NO: 4)
M1-H1 & M1-H3 HCDR3	TGGNYVGWFPY (SEQ ID NO: 5)

M1-L1 & M1-L4 LCDR1	SSVSY (SEQ ID NO: 6)
M1-L1 & M1-L4 LCDR2	DTS (SEQ ID NO: 7)
M1-L1 & M1-L4 LCDR3	QQWSSNPPY (SEQ ID NO: 8)
M8-H3 VH	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGMNWVKQSPGKGL KWMGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYLQISSLKAED TATYFCAHYSFYWGQGTLLTVSS (SEQ ID NO: 37)
M8-L2B2 VL	DIQMTQSPSSLSASVGDVTITCRTSENIYSNLAWYQQKPGKSPQL LVYAATNLADGVPSRFSGSGSGTQYTLKISSLPEDFATYYCQHF WGTPWTFGGGTKVEIK (SEQ ID NO: 38)
M8-L2B5 VL	DIQMTQSPSSLSASVGDRTITCRTSENIYSNLAWYQQKPGKSPQL LVYAATNLADGVPSRFSGSGSGTDYTLTISSLPEDFGTYTCQHF WGTPWTFGGGTKVEIK (SEQ ID NO: 39)
M8-L2B10 VL	DIQMTQSPSSLSASVGDVTITCRTSENIYSNLAWYQQKPGKSPQL LVYAATNLADGVPSRFSGSGSGTDYTLTISSLPEDFGTYTCQHF WGTPWTFGGGTKVEIK (SEQ ID NO: 40)
M8-H3 HCDR1	GTTFTDYG (SEQ ID NO: 41)
M8-H3 HCDR2	INTNTGEP (SEQ ID NO: 20)
M8-H3 HCDR3	AHYSFY (SEQ ID NO: 21)
M8-L2B2, M8- L2B5, & M8- L2B10 LCDR1	ENIYSN (SEQ ID NO: 22)
M8-L2B2, M8- L2B5, & M8- L2B10 LCDR2	AAT (SEQ ID NO: 23)
M8-L2B2, M8- L2B5, & M8- L2B10 LCDR3	QHFWGTPWT (SEQ ID NO: 24)
M43-H1bm5 VH	QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGISWVRQRTGQG LEWIGEIYPRSGNTYYNEKFKGKVTLTDDKSTRAYMELRSLTSE DTAVYYCAGKDYGSTYADYWGQGTLLTVSS (SEQ ID NO: 42)
M43-H4bm5 VH	QVQLQQSGAEVAKPGASVNLNCKASGYTFTSYGISWVRQRTGQG LEWIGEIYPRSGNTYYNEKFKGKATLTADKSTRAYMELRSLTSE DTAVYFCAGKDYGSTYADYWGQGTLLTVSS (SEQ ID NO: 43)

M43-L4 VL	QIVLTQSPATLSASPGERVMTMTCASSSSVSSRYLHWYQQKSGASPR LWIYGTSNLASGVPARFSGSGPGTSYTLTISSVEPEDAATYYCQQY HSDPLTFGQGTKLELK (SEQ ID NO: 44)
M43-H1bm5 & M43-H4bm5 HCDR1	GYTFTSYG (SEQ ID NO: 27)
M43-H1bm5 & M43-H4bm5 HCDR2	IYPRSGNT (SEQ ID NO: 28)
M43-H1bm5 & M43-H4bm5 HCDR3	AGKDYGSTYADY (SEQ ID NO: 29)
M43-L4 LCDR1	SSVSSRY (SEQ ID NO: 30)
M43-L4 LCDR2	GTS (SEQ ID NO: 31)
M43-L4 LCDR3	QQYHSDPLT (SEQ ID NO: 32)

Table 9 – Humanized Antibody Full-Length Amino Acid Sequences

Sequence ID	Sequence
M1-H1 HC	EVQLVQSGAEVKKPGATVKISCTASGFNIKDEYINWVKQRPQKGL EWIGWIDPENGDT EYASKFQGRVTITADTSTD TAYLELSSLRSED AVYYCTGGNYVGWFPYWGQGT LTVSSASTKGPSVFPLAPSSK TSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 45)
M1-H3 HC	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYINWVKQRPQGG LEWIGWIDPENGDT EYASKFQGRATITADTSTD TAYLELSSLRSED TAVYYCTGGNYVGWFPYWGQGT LTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

		K (SEQ ID NO: 46)
M1-L1 LC		EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWYQQKPGTSPRRW IYDTSKLASGVPARFSGSGSGTDFTLTISSELPEDFAVYYCQQWSS NPPYTFGQGTKLEIKTVAAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 47)
M1-L1 variant	LC	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWYQQKPGTSPRRW IYDTSKLASGVPARFSGSGSGTDFTLTISSELPEDFAVYYCQQWSS NPPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 159)
M1-L4 LC		QIVLTQSPATLSASPGERVMTMCSASSSVSYMHWYQQKPGTSPRR WLYDTSKLASGVPARFSGSGSGTDYTLTISSELPEDFATYYCQQW SSNPPYTFGQGTKLEIKTVAAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 48)
M1-L4 variant	LC	QIVLTQSPATLSASPGERVMTMCSASSSVSYMHWYQQKPGTSPRR WLYDTSKLASGVPARFSGSGSGTDYTLTISSELPEDFATYYCQQW SSNPPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 160)
M8-H3 HC		QIQLVQSGSELKKPGASVKISCKASGTTFTDYGMINWVKQSPGKGL KWMGWINTNTGEPTYAEDFKGRFVFLDTSVSTAYLQISSLKAED TATYFCAHYSDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 49)
M8-L2B2 LC		DIQMTQSPSSLSASVGDVTITCRTSENIYSNLAWYQQKPGKSPQL LVYAATNLADGVPSRFSGSGSGTQYTLKISSELPEDFATYYCQHF WGTPWTFGGGKVEIKTVAAAPSVFIFPPSDEQLKSGTASVVCLLNN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 50)
M8-L2B2 Variant	LC	DIQMTQSPSSLSASVGDVTITCRTSENIYSNLAWYQQKPGKSPQL LVYAATNLADGVPSRFSGSGSGTQYTLKISSELPEDFATYYCQHF WGTPWTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLS SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 161)
M8-L2B5 LC		DIQMTQSPSSLSASVGDRTITCRTSENIYSNLAWYQQKPGKSPQL LVYAATNLADGVPSRFSGSGSGTDYTLTISSELPEDFGTYTCQHF WGTPWTFGGGKVEIKTVAAAPSVFIFPPSDEQLKSGTASVVCLLNN

		NFYBPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLS KADYKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 51)
M8-L2B5	LC	DIQMTQSPSSLSASVGDRVTITCRTSENIYSNLAWYQQKPGKSPQL LVYAATNLADGVPSRFSGSGSGTDYTLTISSLQPEDFGTYTCQHF WGTPWTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYBPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLS KADYKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 162)
M8-L2B10	LC	DIQMTQSPSSLSASVGDTVTITCRTSENIYSNLAWYQQKPGKSPQL LVYAATNLADGVPSRFSGSGSGTDYTLTISSLQPEDFGTYTCQHF WGTPWTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYBPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLS KADYKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 52)
M8-L2B10	LC	DIQMTQSPSSLSASVGDTVTITCRTSENIYSNLAWYQQKPGKSPQL LVYAATNLADGVPSRFSGSGSGTDYTLTISSLQPEDFGTYTCQHF WGTPWTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYBPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLS KADYKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 163)
M43-H1bm5	HC	QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGISWVRQRTGQG LEWIGEIYPRSGNTYYNEKFKGKVTLTDDKSTRATAYMELRSLTSE DTAVYYCAGKDYGSTYADYWGQGTTLTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD DGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS PGK (SEQ ID NO: 53)
M43-H4bm5	HC	QVQLQSGAEVAKPGASVNLNCKASGYTFTSYGISWVRQRTGQG LEWIGEIYPRSGNTYYNEKFKGKATLTADKSTRATAYMELRSLTSE DTAVYFCAGKDYGSTYADYWGQGTTLTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK THTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSP GK (SEQ ID NO: 54)
M43-L4	LC	QIVLTQSPATLSASPGERVMTTCSSASSVSSRYLHWYQQKSGASPR LWIYGTSNLAAGVPSRFSGSGPGTSYTLTISSVEPEDAATYYCQQY HSDPLTFGQGTKLELKTVAAPSVFIFPPSDEQLKSGTASVVCLLN FYBPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSK ADYKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 55)

M43-L4 Variant	LC	QIVLTQSPATLSASPGERVMTMCSASSSVSSRYLHWYQQKSGASPR LWIYGTSNLASGVPARFSGSGPGTSYTLTISSVEPEDAATYYCQQY HSDPLTFGQGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLS KADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 164)
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Table 10 – ScFv Antibody Amino Acid Sequences

Sequence ID	Sequence
M1 ScFv (vH1-vL4)	EVQLVQSGAEVKKPGATVKISCTASGFNIKDEYINWVKQRPKGLEW IGWIDPENGDEYASKFQGRVTITADTSTDYALELSSLRSEDTAVYY CTGGNYVGWFPYWGQGTLLTVSSGGGGSGGGGSGGGGSGIVLTQSP ATLSASPGERVMTMCSASSSVSYMHYQQKPGTSPRRWLYDTSKLAS GVPARFSGSGSGTDYTLTISSLEPEDFATYYCQQWSSNPPYTFGQGTK LEIK (SEQ ID NO: 56)
M1 ScFv (vL4-vH1)	QIVLTQSPATLSASPGERVMTMCSASSSVSYMHYQQKPGTSPRRWL YDTSKLASGVPARFSGSGSGTDYTLTISSLEPEDFATYYCQQWSSNPP YTFGQGTKLEIKGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGATVKI SCTASGFNIKDEYINWVKQRPKGLEWIGWIDPENGDEYASKFQGR VTITADTSTDYALELSSLRSEDTAVYYCTGGNYVGWFPYWGQGTLL TVSS (SEQ ID NO: 57)
M1 ScFv (vH3-vL1)	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYINWVKQRPQGGL WIGWIDPENGDEYASKFQGRATITADTSTDYALELSSLRSEDTAVY YCTGGNYVGWFPYWGQGTLLTVSSGGGGSGGGGSGGGGSEIVLTQS PATLSLSPGERATLSCSASSSVSYMHYQQKPGTSPRRWIYDTSKLAS GVPARFSGSGSGTDFTLTISLEPEDFAVYYCQQWSSNPPYTFGQGTK LEIK (SEQ ID NO: 58)
M1 ScFv (vL1-vH3)	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHYQQKPGTSPRRWIY DTSKLASGVPARFSGSGSGTDFTLTISLEPEDFAVYYCQQWSSNPPY TFGQGTKLEIKGGGGSGGGGSGGGGSEVQLQQSGAEVKKPGATVKL SCTASGFNIKDEYINWVKQRPQGGLWIGWIDPENGDEYASKFQGR ATITADTSTDYALELSSLRSEDTAVYYCTGGNYVGWFPYWGQGTLL TVSS (SEQ ID NO: 59)
M1 ScFv (vH3-vL4)	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYINWVKQRPQGGL WIGWIDPENGDEYASKFQGRATITADTSTDYALELSSLRSEDTAVY YCTGGNYVGWFPYWGQGTLLTVSSGGGGSGGGGSGGGGSGIVLTQS PATLSASPGERVMTMCSASSSVSYMHYQQKPGTSPRRWLYDTSKLA SGVPARFSGSGSGTDYTLTISSLEPEDFATYYCQQWSSNPPYTFGQGT KLEIK (SEQ ID NO: 60)
M1 ScFv (vL4-vH3)	QIVLTQSPATLSASPGERVMTMCSASSSVSYMHYQQKPGTSPRRWL YDTSKLASGVPARFSGSGSGTDYTLTISSLEPEDFATYYCQQWSSNPP YTFGQGTKLEIKGGGGSGGGGSGGGGSEVQLQQSGAEVKKPGATVK LSCTASGFNIKDEYINWVKQRPQGGLWIGWIDPENGDEYASKFQGR RATITADTSTDYALELSSLRSEDTAVYYCTGGNYVGWFPYWGQGTLL TVSS (SEQ ID NO: 61)
M8 ScFv	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGMNWVKQSPGKGLK

(vH3-vL2B2)	WMGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYLQISSLKAEDTAT YFCAHYSFDYWGGQTLLTVSSGGGGSGGGGSGGGGSDIQMTQSPSSL SASVGDTVITICRTSENIYSNLAWYQQKPGKSPQLLVYAATNLADGV PSRFSGSGSGTQYTLKISSLQPEDFATYYCQHFWGTPWTFGGGKVEI K (SEQ ID NO: 62)
M8 ScFv (vL2B2-vH3)	DIQMTQSPSSLSASVGDTVITICRTSENIYSNLAWYQQKPGKSPQLLV YAATNLADGVPSRFSGSGSGTQYTLKISSLQPEDFATYYCQHFWGTP WTFGGGKVEIKGGGGSGGGGSGGGGSIQQLVQSGSELKKPGASVKI SCKASGTTFTDYGMNWVKQSPGKGLKWMGWINTNTGEPTYAEDFK GRFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSFDYWGGQTLLTVS S (SEQ ID NO: 63)
M8 ScFv (vH3-vL2B5)	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGMNWVKQSPGKGLK WMGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYLQISSLKAEDTAT YFCAHYSFDYWGGQTLLTVSSGGGGSGGGGSGGGGSDIQMTQSPSSL SASVGDRVITICRTSENIYSNLAWYQQKPGKSPQLLVYAATNLADGV PSRFSGSGSGTDYTLTISSLQPEDFGTYTCQHFWGTPWTFGGGKVEI K (SEQ ID NO: 64)
M8 ScFv (vL2B5-vH3)	DIQMTQSPSSLSASVGDRVITICRTSENIYSNLAWYQQKPGKSPQLLV YAATNLADGVPSRFSGSGSGTDYTLTISSLQPEDFGTYTCQHFWGTP WTFGGGKVEIKGGGGSGGGGSGGGGSIQQLVQSGSELKKPGASVKI SCKASGTTFTDYGMNWVKQSPGKGLKWMGWINTNTGEPTYAEDFK GRFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSFDYWGGQTLLTVS S (SEQ ID NO: 65)
M8 ScFv (vH3- vL2B10)	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGMNWVKQSPGKGLK WMGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYLQISSLKAEDTAT YFCAHYSFDYWGGQTLLTVSSGGGGSGGGGSGGGGSDIQMTQSPSSL SASVGDTVITICRTSENIYSNLAWYQQKPGKSPQLLVYAATNLADGV PSRFSGSGSGTDYTLTISSLQPEDFGTYTCQHFWGTPWTFGGGKVEI K (SEQ ID NO: 66)
M8 ScFv (vL2B10- vH3)	DIQMTQSPSSLSASVGDTVITICRTSENIYSNLAWYQQKPGKSPQLLV YAATNLADGVPSRFSGSGSGTDYTLTISSLQPEDFGTYTCQHFWGTP WTFGGGKVEIKGGGGSGGGGSGGGGSIQQLVQSGSELKKPGASVKI SCKASGTTFTDYGMNWVKQSPGKGLKWMGWINTNTGEPTYAEDFK GRFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSFDYWGGQTLLTVS S (SEQ ID NO: 67)
M43 ScFv (vH1-vL4)	QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGISWVRQRTGQGLE WIGEYPRSGNTYYNEKFKGVTLTTDKSTRTAYMELRSLTSEDNAV YYCAGKDYGSTYADYWGGQTLLTVSSGGGGSGGGGSGGGGSIQVLT QSPATLSASPGERVMTCSASSSVSSRYLHWYQQKSGASPRLWIYGTS NLASGVPARFSGSGPGTSYTLTISSVEPEDAATYYCQQYHSDPLTFGQ GTKLELK (SEQ ID NO: 68)
M43 ScFv (vL4-vH1)	QIVLTQSPATLSASPGERVMTCSASSSVSSRYLHWYQQKSGASPRL WIYGTSNLASGVPARFSGSGPGTSYTLTISSVEPEDAATYYCQQYHSD PLTFGQGTKLELKGGGGSGGGGSGGGGSIQVQLVQSGAEVAKPGASV NLNCKASGYTFTSYGISWVRQRTGQGLEWIGEYPRSGNTYYNEKFK GKVTLLTTDKSTRTAYMELRSLTSEDNAVYYCAGKDYGSTYADYWG

	QGTTLTVSS (SEQ ID NO: 69)
M43 ScFv (vH4-vL4)	QVQLQQSGAEVAKPGASVNLNCKASGYTFTSYGISWVRQRTGQGLE WIGEYPRSGNTYYNEKFKGKATLTADKSTRTAYMELRSLTSED TAVYFCAGKDYGSTYADYWGQGTTLTVSSGGGGSGGGSGGGGSQIVLT QSPATLSASPGERVMTMCSASSSVSSRYLHWYQQKSGASPRLWIYGT SNLASGVPARFSGSGPGTSYTLTISSVEPEDAATYYCQQYHSDPLTFGQ GTKLELK (SEQ ID NO: 70)
M43 ScFv (vL4-vH4)	QIVLTQSPATLSASPGERVMTMCSASSSVSSRYLHWYQQKSGASPRL WIYGTSNLASGVPARFSGSGPGTSYTLTISSVEPEDAATYYCQQYHSD PLTFGQGTKLELKGGGGSGGGSGGGGSQVQLQQSGAEVAKPGASV NLNCKASGYTFTSYGISWVRQRTGQGLEWIGEYPRSGNTYYNEKFK GKATLTADKSTRTAYMELRSLTSED TAVYFCAGKDYGSTYADYWGQ GTTLTVSS (SEQ ID NO: 71)

The binding affinity of the humanized anti-uPAR antibodies M1, M8, and M43 was determined. Binding affinity analysis was carried out using the Octet platform as described above. Briefly, uPAR antibodies were immobilized via an Anti-human Fc (AHC) sensor. Each uPAR antibody was tested at a concentration of 10 µg/mL. The load was histidine-tagged human uPAR provided over a titration starting at 50 nM with subsequent 2-fold dilutions. The binding affinities of the tested humanized antibodies against human uPAR are recited below in **Table 11**. Notably, the humanized antibodies retained similar binding affinity to human uPAR compared to the parental antibodies.

Table 11 – Humanized Antibody Binding Affinity Data

Antibody	KD (M)	k on(1/Ms)	k dis(1/s)
M1 – Chimeric	1.11E-09	6.29E+05	7.01E-04
M1 – H1/L4	1.48E-09	6.65E+05	9.86E-04
M1 – H3/L1	1.64E-09	6.77E+05	1.11E-03
M1 – H3/L4	1.30E-09	6.86E+05	8.93E-04
M8 – Chimeric	1.79E-09	2.73E+05	4.90E-04
M8 – H3/L2B2	1.74E-09	2.46E+05	4.27E-04
M8 – H3/L2B5	1.65E-09	2.13E+05	3.51E-04
M8 – H3/L2B10	2.35E-09	2.03E+05	4.77E-04
M43 – Chimeric	1.51E-08	4.02E+05	6.06E-03
M43 – H1bm5/L4	1.59E-08	4.15E+05	6.58E-03
M43 – H4bm5/L4	1.47E-08	4.54E+05	6.66E-03

Example 3 – Interleukin 12 (IL-12) Engineering

IL-12 is a heterodimeric cytokine composed of a p35 subunit and a p40 subunit, which associate to form IL-12p70. IL-12 is expressed by antigen presenting cells and plays multiple roles in the immuno-oncology lifecycle. IL-12 is a potent stimulator of the immune response, but toxicity issues have limited IL-12 use in the clinic. Localized delivery may reduce toxicity, but this precludes systemic delivery.

Engineering one or both of the p35 subunit and p40 subunit offers several advantages. A “Lock and Key” set of mutations can be made in each subunit to modulate affinity and enhance specificity between the two subunits. For example, mutations introduced into the engineered subunits may reduce recognition to the wild-type subunits, further reducing toxicity. Paired charge residues at the interface between p35 and p40 may be engineered to introduce specificity further specificity.

IL12p35 / IL12p40 Cysteine Residue Engineering

As part of an initial optimization strategy, several point mutations were identified in p35 and p40 to improve stability and expression. An intermolecular disulfide bridge is formed between C74 of p35 and C177 of p40. A screen was performed with amino acid substitutions at C74 of p35 and C177 of p40 to eliminate the disulfide bridge, while retaining or improving binding affinity between the two subunits. For p35, substitutions C74S, S(SS1), D, K, R, Q, W, and Y were generated. For p40, substitutions C177S, H, M, F, Y, R, K, E, Q, I, W, and N were generated. S(SS1) corresponds to a C15S, C88S, and C74S substitution in p35. The C15S / C88S pair removes internal disulfide bridge. Each p35 C74 variant was then tested against each of the p40 C177 variants. Binding affinity was measure using the Octet platform described above. Briefly, a sensor was loaded with 5-10 µg/mL of either p35 or p40. A capture compound was provided at a concentration range of 500 nM to 7.8 nM. As shown in **FIG. 3**, several p35 / p40 variant pairs demonstrated high binding affinity.

Select p35 / p40 variant pairs were chosen for further binding affinity characterization. Specifically, a p35 C74D, K, W, and Y variant was paired with either a p40 C177S or C177F variant. The full binding affinity data is recited below in **Tables 12** and **13**. As shown below, each p35 / p40 variant pair displayed high binding affinity.

Table 12 – p40 C177S Binding Affinity To p35 C74D, K, W, and Y

P35 C74 Sub.	KD (M)	KD error	Kon (1/Ms)	Kon error	Kdis (1/s)	Kdis error
D	5.71E-08	3.92E-10	1.76E+04	1.05E+02	1.00E-03	3.36E-06
K	1.96E-08	1.39E-10	2.59E+04	1.18E+02	5.08E-04	2.78E-06
W	2.70E-08	2.49E-10	1.60E+04	9.85E+01	4.32E-04	2.97E-06
Y	2.32E-08	2.13E-10	1.77E+04	1.03E+02	4.11E-04	2.91E-06

Table 13 – p40 C177F Binding Affinity To p35 C74D, K, W, and Y

P35 C74 Sub.	KD (M)	KD error	Kon (1/Ms)	Kon error	Kdis (1/s)	Kdis error
D	6.02E-08	5.34E-10	1.19E+04	9.13E+01	7.19E-04	3.23E-06
K	1.84E-08	1.34E-10	1.60E+04	6.15E+01	2.95E-04	1.82E-06
W	2.54E-08	2.15E-10	1.15E+04	5.85E+01	2.91E-04	1.96E-06
Y	2.38E-08	2.69E-10	1.25E+04	8.43E+01	2.98E-04	2.71E-06

5 In addition to the above recited disulfide bridge substitutions, a free thiol was identified in p40 (C252). This site was also engineered to a C252S substitution to remove the free thiol

IL12p35 / IL12p40 Engineering – Stability Enhancement

10 While linking p35 and p40 to fusion partners can improve stability and production yield (see below), it would be advantageous to engineer the subunits to improve stability without a fusion partner. p35 in particular is unstable on its own, being aided significantly by a fusion partner or p40 for proper folding and expression.

At the outset, S27D and D188N mutations in p35 were identified via a directed molecular evolution approach (see, Leong et al. PNAS. 100(3): 1163-1168. 2003). The p35 substations are useful for increased yield.

Several surface exposed hydrophobic regions and flexible regions were identified on p35. An *in silico* modeling and simulation approach was used to identify potential amino acid mutations (i.e., substitutions, deletions, and/or insertions) that can improve p35 stability. As a first pass, 85 single point mutations were ranked by delta-delta G (ddG, i.e., the difference between delta G for the WT and mutant p35), solubility, position-specific scoring matrix (pssm) scores, and epitope prediction. The process attempted to introduce polar residues in place of solvent-exposed hydrophobic residues for better solubility and introduce bulky hydrophobic residues internally for better core-packing. Each single mutant was tested for expression and stability through western blot. Following western blot analysis, proteins were purified and tested by HPLC to determine % monomer and % aggregation. The point mutations tested by western blot are recited below in **Table 14**. Sample ID numbers 70-90 were p35-murine serum albumin (MSA) fusions. Sample ID numbers in bold italicized text (ID 72, 75, 76, 80, 82, 83) displayed the best expression among those tested, although all p35-MSA fusions expressed.

Table 14 – Point Mutation(s) Tested For Expression By Western Blot

Sample ID	Point Mutation(s)	Sample ID	Point Mutation(s)
1	M119L	49	R1D
2	M145L	50	N2D
3	M111L	51	A6E
4	M119Y	52	A6H
5	F150Y	53	A6Q
6	M111W	54	F39E
7	V114I	55	F39Y
8	M111F	56	F39H
9	M12R	57	F39K
10	M12S	58	F39N
11	S27D	59	S44E
12	Q35E / N28R	60	K117E
13	Q35E	61	E143R
14	S44K	62	N151D
15	V60E	63	S159Q
16	T80K	64	S159R

17	T80R	65	S81C / A99C
18	K117R	66	T59C / S104C
19	N120D	67	T80C / S103C
20	K128Q	68	M29C / N136C
21	N136R	69	WT
22	A139T	70	WT-MSA
23	L161D	71	M12R
24	L161E	72	<i>M12S</i>
25	L161K	73	V60E
26	L161N	74	M111L
27	L161Q	75	<i>M111F</i>
28	L161S	76	<i>M111W</i>
29	K172R	77	M119L
30	S192N	78	M119Y
31	A121K	79	V114I
32	M12Q	80	<i>M145L</i>
33	S27E	81	F150Y
34	N28R	82	<i>L161D</i>
35	T80N	83	<i>L161Q</i>
36	T80Q	84	L161S
37	A121N	85	F39D
38	K122R	86	F39H
39	A139Q	87	S27D
40	I171V	88	Q35E / N28R
41	N21D	89	T80K
42	R24E	90	S44K
43	S27H		
44	F39D		
45	S103N		
46	A139R		
47	F132Y		
48	S159E		

Following the western blot analysis, sample ID numbers 69-90 (p35-MSA fusions) were tested by HPLC to determine % monomer and % aggregate. The results are depicted below in **Table 15**, which shows that several p35 point mutants enjoyed greater % monomer content relative to the WT p35.

Table 15 – Point Mutation(s) Tested For % Monomer And % Aggregate Content

Mutant	Monomer %	Aggregate %
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WT (no MSA)	0	100
WT-MSA	5.84	94.16
M12R	3.92	96.08
M12S	5.36	94.64
V60E	4.35	95.65
M111L	0	100
M111F	8.88	91.12
M111W	9.73	90.27
M119L	5.55	94.45
M119Y	0	100
V114I	5.57	94.43
M145L	7.98	92.02
F150Y	5.97	94.03
L161D	5.67	94.33
L161Q	5.25	94.75
L161S	4.65	95.35
F39D	7.43	92.57
F39H	6.2	93.8
S27D	6.47	93.53
Q35E / N28R	7.1	92.9
T80K	0	100
S44K	7.59	92.41

The results of the single point mutant screen revealed several point mutations that improve p35 expression and stability. In particular, the following single p35 mutants displayed % monomer values that were similar to or exceeded the value for the WT p35-MSA fusion:

5 M12S, S27D, F39D, F39H, S44K, M111F, M111W, V114I, M119L, M145L, F150Y, L161D, L161Q.

A double mutant, Q35E / N28R, also displayed % monomer value that exceeded the value for the WT p35-MSA fusion.

Double mutations will be tested to further improve these parameters.

5 IL12p35 / IL12p40 Fusions

IL-12 subunits p35 and p40 present several manufacturing challenges that should be overcome before IL-12 can be used as a viable therapeutic. p35 shows little to no expression. Expression can be improved by linking p35 to a fusion partner, such as an antibody or fragment thereof, or human serum albumin (HSA). p35 is also prone to aggregation. p40 shows similar challenges. p40 can be expressed without a fusion partner, but the purified composition is frequently contaminated with a p40 homodimer (about 15-20%). p40 is also prone to dimerization and aggregation over time at 37 °C.

The p35 and p40 subunits were tested for stability at 37 °C with various fusion partners. p40 was tested with an antibody Fab fusion partner and a full-length IgG fusion partner, with two p40 subunits linked to each CH3 domain of the IgG molecule. The Fab and full-length antibodies were derived from anti-uPAR antibodies described above. p35 was tested with a mouse serum albumin (MSA) fusion partner and an CH1-hinge-CH2-CH3 antibody fragment fusion partner. A single chain IL-12 (scIL-12), where the p35 and p40 subunits are linked with an amino acid linker, was also tested. Exemplary IL-12 subunit fusion proteins are depicted in **FIG. 4**. Each fusion protein was tested by HPLC analysis to measure aggregate and dimer formation at 37 °C for 0 hours, 24 hours, 48 hours, 1 week, and 2 weeks. The results for each fusion protein are recited below in **Table 16** to **Table 21**. The results demonstrate that linking the p35 and p40 subunits to various fusion partners can reduce aggregate and homodimer formation.

25

Table 16 – p40 subunit alone. Predicted Molecular Weight (Da) – 37087. Bold values indicate the monomer. Other values represent aggregates and dimers. Differences in the predicted MW and calculated MW are due, in part, to protein glycosylation.

Time point	Calculated MW (Da)	interpolated Y value	Elution time (Vt)	%Area
0 hours	97198	4.988	4.601	19.1

	53816	4.731	5.090	80.9
24 hours	1091167	6.038	3.051	9
	94812	4.977	4.589	56
	52814	4.723	5.070	35
48 hours	1255456	6.099	3.017	13
	96647	4.985	4.603	58
	54541	4.737	5.086	29
1 week	1793115	6.254	2.909	6
	97156	4.987	4.607	61
	54692	4.738	5.097	33
2 week	95072	4.978	4.637	60
	52744	4.722	5.134	40

Table 17 – p40-Fab fusion. Predicted Molecular Weight (Da) – 62310. Bold values indicate the monomer. Other values represent aggregates and dimers. Differences in the predicted MW and calculated MW are due, in part, to protein glycosylation.

Time point	Calculated MW (Da)	interpolated Y value	Elution time (Vt)	%Area
0 hours	171495	5.234	4.068	3.1
	94351	4.975	4.629	96.9
24 hours	908794	5.958	3.116	5
	165574	5.219	4.065	15
	91339	4.961	4.624	80
48 hours	1000083	6.000	3.095	57
	165168	5.218	4.089	14
	92123	4.964	4.649	29
1 week	1055017	6.023	3.080	81
	167051	5.223	4.084	6
	93333	4.970	4.646	13
2 week	1070333	6.030	3.087	93
	168418	5.226	4.085	2

	90472	4.957	4.685	5
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Table 18 – p40-IgG fusion. Predicted Molecular Weight (Da) – 215845. Bold values indicate the monomer. Other values represent aggregates and dimers. Differences in the predicted MW and calculated MW are due, in part, to protein glycosylation.

Time point	Calculated MW (Da)	interpolated Y value	Elution time (Vt)	%Area
0 hours	246412	5.392	3.787	100
24 hours	803801	5.905	3.162	4
	239288	5.379	3.779	96
48 hours	912829	5.960	3.128	10
	240573	5.381	3.793	90
1 week	1114509	6.047	3.061	69
	242653	5.385	3.791	31
2 week	1179011	6.072	3.054	93
	237524	5.376	3.815	7

5

Table 19 – p35-MSA fusion. Predicted Molecular Weight (Da) – 92118. Bold values indicate the monomer. Other values represent aggregates and dimers. Differences in the predicted MW and calculated MW are due, in part, to protein glycosylation.

Time point	Calculated MW (Da)	interpolated Y value	Elution time (Vt)	%Area
0 hours	115158	5.061	4.438	100
24 hours	113407	5.055	4.417	100
48 hours	757199	5.879	3.199	9
	253144	5.403	3.758	2
	113751	5.056	4.443	90
1 week	855333	5.932	3.156	21
	257674	5.411	3.750	4
	115312	5.062	4.437	75
2 week	870909	5.940	3.161	51
	116025	5.065	4.439	49

Table 20 – p35-CH1-hinge-CH2-CH3 antibody fragment fusion. Predicted Molecular Weight (Da) – 124339. Bold values indicate the monomer. Other values represent aggregates and dimers. Differences in the predicted MW and calculated MW are due, in part, to protein glycosylation.

Time point	Calculated MW (Da)	interpolated Y value	Elution time (Vt)	%Area
0 hours	150142	5.177	4.186	100
24 hours	791345	5.898	3.168	11
	145363	5.162	4.181	89
48 hours	842195	5.925	3.158	22
	145523	5.163	4.204	78
1 week	935179	5.971	3.123	44
	147343	5.168	4.198	56
2 week	924568	5.966	3.139	62
	143473	5.157	4.231	38

Table 21 – scIL-12. Predicted Molecular Weight (Da) – 61001. Bold values indicate the monomer. Other values represent aggregates and dimers. Differences in the predicted MW and calculated MW are due, in part, to protein glycosylation.

Time point	Calculated MW (Da)	interpolated Y value	Elution time (Vt)	%Area
0 hours	104159	5.018	4.535	100
24 hours	102149	5.009	4.518	100
48 hours	103206	5.014	4.539	100
1 week	1013855	6.006	3.094	3
	105574	5.024	4.525	97
2 week	1013733	6.006	3.106	4
	102693	5.012	4.561	96

10

The p35-MSA fusion protein and p40-fab fusion protein were next tested for binding affinity to each other over time and at 37 °C. Using the Octet platform described above,

biotinylated p35-MSA was immobilized at a concentration range of 50 nM to 0.78 nM with p40-fab as the load at a concentration of 10-20 µg/mL. The binding affinity data at 0 hours, 4 hours, 24 hours, and 47 hours is reported below in **Table 22**. The results show that p35 / p40 affinity remains high even after 47 hours at 37 °C.

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Table 22 – p35-MSA / p40-fab Binding Affinity at 37 °C

Time	KD (M)	KD Error	k on(1/Ms)	k on Error	k dis(1/s)	k dis Error	Full R ²
0hr	7.82E-09	4.78E-11	5.91E+04	2.15E+02	4.62E-04	2.27E-06	0.999
4hr	7.54E-09	5.34E-11	5.11E+04	1.94E+02	3.85E-04	2.30E-06	0.999
24hr	1.11E-08	6.70E-11	3.81E+04	1.32E+02	4.22E-04	2.10E-06	0.999
47hr	1.05E-08	8.68E-11	3.58E+04	1.60E+02	3.76E-04	2.61E-06	0.999

Example 4 – Split IL-12 Activity

The therapeutic benefit of IL-12 is at least in part achieved when the p35 subunit and a p40 subunit are able to heterodimerize to form IL-12p70. To that end, the ability of each subunit with different fusion partners to heterodimerize was tested. The fusion protein combinations tested are recited below in **Table 23**.

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Table 23 – IL-12 Subunit Combinations

Combination Number	Description
1	Human scIL-12
2	p35-MSA + p40 without a fusion partner
3	p35-MSA + p40-Fab (anti-uPAR M37 Fab)
4	p35-MSA + p40-IgG (anti-uPAR M37 IgG)
5	p35-MSA + p40 with a E181R amino acid substitution
6	p35-CH1-HINGE-CH2-CH3 antibody fragment (anti-uPAR M37 KiH / p35 KiH pair) + p40 without a fusion partner
7	p35-CH1-HINGE-CH2-CH3 antibody fragment (anti-uPAR M37 KiH / p35 KiH pair) + p40 with a E181R amino acid substitution

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The above recited combinations were tested in a cell-based *in vitro* IL-12 activity assay. Briefly, frozen T cells (cultured with rhIL-2 + CD3/CD28 for 9 days prior to assay start) were thawed, seeded at 300,000 cells/well and rested for 1 hour at 37 °C. The IL-12 subunits were added to T cells at a 1:1 ratio (for a final starting concentration of each molecule at 250 nM). Phosphorylated Stat4 (pStat4) and IFN gamma were then detected. For

20

the pStat4 detection assay, cells and IL-12 subunits were incubated for 45 minutes at 37 °C. For the IFN gamma detection assay, cells and IL-12 subunits were incubated for 24 hours at 37 °C prior to collection of supernatant.

Each combination was tested for the ability to induce Stat4 phosphorylation in CD8+ (FIG. 6A) and CD4+ (FIG. 6B) T cells. Briefly, cells from each sample were fixed and incubated with an anti-pStat4 antibody labeled with the fluorophore phycoerythrin (PE). Fluorescence was then detected. The EC50 values and EC50 fold change relative to scIL-12 are recited below in Table 24 for the CD8+ T cells and Table 25 for the CD4+ T cells.

Table 24 – EC50 values for % pStat4 Formation In CD8+ T cells

	scIL-12	p35-MSA + p40-Fab	p35-MSA + p40-IgG	p35 KIH + p40	p35-MSA + p40
EC50 (nM)	0.0746	0.912	0.5714	1.419	1.060
Fold Change	1	12.23	7.66	19.02	14.21

Table 25 – EC50 values for % pStat4 Formation In CD4+ T cells

	scIL-12	p35-MSA + p40-Fab	p35-MSA + p40-IgG	p35 KIH + p40	p35-MSA + p40
EC50 (nM)	.05115	0.7081	0.1726	1.159	0.8415
Fold Change	1	13.84	3.37	22.66	16.45

Each combination was tested for the ability to induce IFN gamma production in cells as well (FIG. 7). The MSD T-Plex Cytokine Assay system was employed. The MSD assay is an ELISA-based assay, where the MSD plate is pre-coated with a capture antibody to IFN gamma. After incubating the sample on the plate, a labeled detection antibody that also binds

IFN gamma was employed for chemiluminescent detection. The EC50 values and EC50 fold change relative to scIL-12 are recited below in **Table 26**.

Table 26 – EC50 values for IFN gamma production

	scIL-12	p35-MSA + p40-Fab	p35-MSA + p40-IgG	p35 KIH + p40	p35-MSA + p40
EC50 (nM)	0.00515	0.5591	~0.4029	~0.4274	0.6572
Fold Change	1	108.54	78.23	82.99	127.61

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Example 5 – Anti-uPAR / IL-12 Subunit Fusions

10 To enhance the therapeutic potency of IL-12, while reducing toxicity issues, the IL-12 subunits (p35 and p40) were linked to the parental anti-uPAR antibodies described above. The uPAR antibody-IL-12 subunit fusions can deliver the subunits to the target uPAR-expressing tissue or cell (e.g., tumor tissue or cell), thereby ensuring the potent IL-12 activity is only restored at the target site. To that end, the ability of each subunit with different uPAR
15 antibody fusion partners to heterodimerize was tested. The fusion protein combinations tested and the binding affinities between p35 and p40 are recited below in **Table 27**. The binding affinity between the uPAR antibody fusions to uPAR (uPAR-his and uPAR-Fc) is also recited below.

20 Table 27 – Binding Affinities For uPAR Antibody-IL-12 Subunit Fusions

Combination	KD (M)	KD Error	kon(1/Ms)	kon Error	kdis(1/s)	kdis Error	Full R ²
4376 (p35-M1-scFv-KiH) + 4339 (M8-Fab-	6.98e-9	2.45e-10	1.95e+5	6.08e+3	1.36e-3	2.17e-5	0.934786

p40)							
4379 (p35-M1-fab-KiH) + 4339 (M8-Fab-p40)	6.84e-9	6.86e-11	8.56e+4	5.93e+2	5.85e-4	4.25e-6	0.995916
4376 (p35-M1-scFv-KiH) + 4336 (M8-IgG-p40)	4.68e-9	8.43e-11	6.00e+4	4.58e+2	2.81e-4	4.58e-6	0.996298
4379 (p35-M1-fab-KiH) + 4336 (M8-IgG-p40)*	<1.0E-12						0.998201
4398 (p35-M1-scFv2) + 4269 (p40)	2.09e-8	2.21e-10	1.57e+4	1.22e+2	3.27e-4	2.34e-6	0.999902
4376 (p35-M1-scFv-KiH) + uPAR-his	1.49e-10	2.11e-12	2.66e+6	3.28e+4	3.96e-4	2.77e-6	0.98057
4379 (p35-M1-fab-KiH) + uPAR-his	2.29e-10	5.79e-12	3.68e+6	8.89e+4	8.45e-4	6.18e-6	0.944841
4339 (M8-Fab-p40) + uPAR-Fc	1.72e-10	1.38e-12	1.69e+6	1.06e+4	2.91e-4	1.45e-6	0.994733
4398 (p35-M1-scFv2) + uPAR-Fc	1.33e-10	1.94e-12	1.52e+6	1.48e+4	2.02e-4	2.19e-6	0.984586

*No affinity value obtained due to avidity of IgG molecule

Select fusion proteins were next tested with *in vivo* mouse tumor models to show tumor and serum pharmacokinetics from PET/CT imaging of radiolabeled tumor-targeted IL-12 subunits p40 (**FIG. 5A**) and p35 (**FIG. 5B**). Tumor-targeted subunits were measured in the tumor and blood of mice over time, and the % of maximum exposure was determined. The tumor targeted p40 was a fusion of the anti-uPAR antibody M37 in a Fab format with

p40. The tumor targeted p35 was a fusion of the anti-uPAR antibody M37 in a KiH format with p35. Briefly, CT26 tumor bearing BALB/c mice were administered the zirconium-89 (⁸⁹Zr)-labelled fusion proteins at molar mass of 15.5 – 16.9 nmol/kg (target: 16.4 nmol/kg) and a radioactive dose of 182 to 192 μ Ci in 200 μ L, injected intravenously (tail vein). The mice were subsequently imaged via PET/CT at 4 h, 24 h, 48 h, 72 h, and 168 h post injection. At each imaging time point, blood was collected for gamma counting.

Tumor clearance of targeted IL-12 subunits was slower than serum clearance, facilitating tumor-specific assembly.

The above recited fusion proteins were also tested with *in vivo* mouse tumor models to show tumor and liver accumulation. Three anti-uPAR antibody fusion proteins were used as tumor targeted compounds and compared against non-targeted scIL-12 or a p35-MSA fusion protein. The three targeted fusion proteins were Fab-p40, IgG-p40, and p35-KiH. Each compound was radiolabeled to track tissue accumulation in the mouse. The assay was performed as above with ⁸⁹Zr-labelled fusion proteins. As shown in **FIG. 8A**, targeted compounds accumulated in the tumor before beginning to dissipate over time. The untargeted compounds failed to accumulate. **FIG. 8B** shows liver accumulation over time, demonstrate progressive clearance of the compounds. The targeted compounds were cleared at a slower rate, indicating better retention compared to the untargeted compounds.

The fusions were then tested in a humanized mouse model to test for *in vivo* activity of sequentially administered human IL-12 subunits. Immune deficient NCG mice were humanized by injection of 1×10^7 human PBMCs. 14 days later mice were treated with PBS (vehicle), scIL-12, or two doses of split IL-12 molecules. For the split IL-12 molecules, p35-MSA was administered with p40-Fab. scIL-12 was administered at 0.24 mg/kg, while the split IL-12 molecules were administered at 0.6 mg/kg each (2.5x the dose of scIL-12) and 6 mg/kg each (25x the dose of scIL-12). The interval between injections was less than 5 minutes. Mice were then sampled for serum at various timepoints for serum pharmacokinetic and pharmacodynamic (serum IFN gamma concentration) analysis. The data shows that the split fusion molecules function *in vivo*. The data also shows a similar pharmacodynamic response between single chain and split molecules such that in the following experiments one can differentiate between the serum and tumor activity of these treatments (**FIG. 9**). Notably,

a dose that is 2.5x that of the scIL-12 lead to less IFN gamma production, indicating reduced off-target toxicity issues with the split IL-12 fusions. This data further demonstrates the ability of the split IL-12 molecules in assemble *in vivo*.

Additional fusion proteins were tested, along with the potential of dual targeting (i.e.,
5 an antibody-p35 fusion and an antibody-p40 fusion, where each antibody binds different epitopes on the same target protein). The IL-12 subunits were attached to antibody variants M1 and M8 that bind to human uPAR. In this assay, the IL-12p70 complex is built by sequentially adding, followed by washing of the various components to human uPAR positive HEK cells. The uPAR-expressing HEK cells were then incubated with CD8+ T cells and
10 pSTAT4 activity on said T cells was determined. The M37 antibody was used as an untargeted control, as this antibody binds to mouse uPAR and do not cross react with human uPAR. scIL-12 was used as an untargeted single compound. scIL12-M8 Fab fusion was used as a targeted single compound. M37-p40-Fab and M37-p35-KiH were used in combination as an untargeted negative control. M8-p40-Fab and M37-p35-KiH were used in combination as a
15 single targeted combination (i.e., only one of the IL-12 subunits is targeted to uPAR). M8-p40-Fab and M1-p35-KiH-Fab were used in combination as a dual targeted combination (i.e., both of the IL-12 subunits, p35 and p40, are targeted to uPAR). Briefly, the assay was carried out with the following protocol:

- 1) 50,000 uPAR expressing HEK cells were seeded per well in 96-well U bottom, low
20 attachment/binding plates (Corning Product # 4520).
- 2) Plates were spun down, and cells were re-suspended with 100 µL of a titration of the first molecule.
- 3) Cells and the first molecule were incubated for 30 minutes at 4 °C.
- 4) Cells were spun down and washed twice.
- 25 5) Cells were re-suspended with 100 µL of a titration of the second molecule.
- 6) Cells and molecule incubated for 30 minutes at 4 °C.
- 7) Cells were spun down and washed three times.
- 8) Previously frozen human T cells (rested for 1 hour at 37 °C) were added to wells at 100,000 cells per well.
- 30 9) Plates were placed in the incubator for 1 hour (37 °C).
- 10) Plates were spun down, washed, and downstream staining protocols began.

After the T cell incubation step, pSTAT4 activity on said T cells was determined.

Untargeted subunits or untargeted scIL-12 showed reduced activity compared to targeted subunits. The single targeted combination showed enhanced activity relative to all untargeted approaches. Finally, the dual targeted subunits have improved activity compared to an individually targeted pair and recover most of the activity compared to targeted scIL-12, while enjoying reduced systemic toxicity (**FIG. 10**).

The above recited fusion proteins were generated with the parental anti-uPAR antibodies. Similar fusion proteins with the humanized anti-uPAR antibodies were also generated. The amino acid sequences for M1 fusions are recited below in **Table 28**. The amino acid sequences for M8 fusions are recited below in **Table 29**. The amino acid sequences for M43 fusions are recited below in **Table 30**.

Table 28 – Humanized M1 antibody-IL-12 subunit fusion amino acid sequences

Description	Sequence	Notes
hM1-IgG1(H1)-scIL12	EVQLVQSGAEVKKPGATVKISCTASGFNIKDEYIN WVKQRPGKGLEWIGWIDPENGDTEYASKFQGRV TITADTSTDATAYLELSSLRSEDTAVYYCTGGNYV GWFPYWGQGTLLTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGKGGGSGGGSGGGSGGGSGGGSGIWEK KDVYVVELDWYPDAPGEMVVLTCDTPEEDGITW TLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGG EVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTF RCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSS DPQGVTCGAATLSAERVGRDNKEYEYSVEQCED SACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRD IIPDPKPNLQLKPLKNSRQVEVSWEYPTDWSTPH SYFSLTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYSSSSWSEWASVPCSGSGGG GSGGRNLPVATPDPMFPLHHSQNLRLRAVSNM	humanized M1, p40- p35 as scIL-12

	LQKARQTLEFYPTCTSEEIDHEDITKDKTSTVEACL PLELTKNESCLNSRETSFITNGSCLASRKTSFMMA LCLSSYEDLKMYQVEFKTMNAKLLMDPKRQIFL DQNMLAVIDELMQALNFNSETVPQKSSLEEPDFY KTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 165)	
hM1-Fab(H1)- scIL12	EVQLVQSGAEVKKPGATVKISCTASGFNIKDEYIN WVKQRPQGLEWIGWIDPENGDEYASKFQGRV TITADTSTDYALELSSLRSEDYAVYYCTGGNYV GWFPYWGQGTLLTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSVH TFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCGGGSGGGGSIWELKK DVYVVELDWYDPAGEMVVLTCDTPEEDGITWT LDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGE VLSHSLLLHKKEDGIWSTDILKDQKEPKNTFLR CEAKNYSGRFTCWWTITSTDLTFSVKSSRGSSDP QGVTCGAATLSAERVGRDNKEYEYSVEQEDSA CPAAEESLPIEVMVDAVHKLKYENYTSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPTWSTPHSY FSLTFCVQVQGKSKREKKDRVFTDKTSATVICRK NASISVRAQDRYYSSSWSEWASVPCSGGSGGGGS GGRNLPVATPDPMFPCLLHHSQNLLRAVSNNMLQ KARQTLEFYPTCTSEEIDHEDITKDKTSTVEACLPL ELTKNESCLNSRETSFITNGSCLASRKTSFMMA LCLSSYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQ NMLAVIDELMQALNFNSETVPQKSSLEEPDFYKT KIKLCILLHAFRIRAVTIDRVMSYLNASGGSGGHH HHHHGGSGLNDIFEAQKIEWHE (SEQ ID NO: 166)	humanized M1, p40- p35, 6his avi
hM1-IgG1(H3)- scIL12	EVQLQSGAEVKKPGATVKLSCTASGFNIKDEYI NWVKQRPQGLEWIGWIDPENGDEYASKFQGR ATITADTSTDYALELSSLRSEDYAVYYCTGGNY VGWFPYWGQGTLLTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSQSVMHENHNYTQ KSLSLSPGKGGGSGGGGSIWELKKDVYVVELD WYDPAGEMVVLTCDTPEEDGITWTLDSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSSHLL LHKKEDGIWSTDILKDQKEPKNTFLRCEAKNYS GRFTCWWTITSTDLTFSVKSSRGSSDPQGVTCG AATLSAERVGRDNKEYEYSVEQEDSACPAEES LPIEVMVDAVHKLKYENYTSFFIRDIKPDPPKNL QLKPLKNSRQVEVSWEYPTWSTPHSYFSLTFCV VQVQGKSKREKKDRVFTDKTSATVICRKNASISVR AQDRYYSSSWSEWASVPCSGGSGGGGSGGRNLP VATPDPMFPCLLHHSQNLLRAVSNNMLQKARQTL EFYPTCTSEEIDHEDITKDKTSTVEACLPLELTKNES CLNSRETSFITNGSCLASRKTSFMMA LCLSSYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVI	humanized M1, p40- p35

	DELMQALNFNSETVPQKSSLEEDFYKTKIKLCIL LHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 167)	
hM1-Fab(H3)- scIL12	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYI NWVKQRPQGGLWIGWIDPENGDEYASKFQGR ATITADTSTDATAYLELSSLRSEDATVYYCTGGNY VGWFPYWGQGLTVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCGGGSGGGGSIWELK KDVYVVELDWYPDAPGEMVVLTCDTPEEDGITW TLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGG EVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTF RCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSS DPQGVTCGAATLSAERVRGDNKEYEYSVEQED SACPAAEESLPIEVMVDAVHKLKYENYTSFFIRD IHKPDPKKNLQKPLKNSRQVEVSWEYPDTWSTPH SYFSLTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGGSGGG GSGGRNLPVATPDGPMFPCLLHHSQNLRLRAVSNM LQKARQTLFYPCTSEEIDHEDITDKTSTVEACL PLELTKNESCLNSRETSFITNGSCLASRKTSFMMA LCLSSIEDLKMYQVEFKTMNAKLLMDPKRQIFL DQNMLAVIDELMQALNFNSETVPQKSSLEEDFY KTKIKLCILLHAFRIRAVTIDRVMSYLNASGGSGG HHHHHHGGSGGLNDIFEAKIEWHE (SEQ ID NO: 168)	humanized M1, p40- p35, 6his avi
hM1-IgG1(H1)- p40	EVQLVQSGAEVKKPGATVKISCTASGFNIKDEYIN WVKQRPKGGLWIGWIDPENGDEYASKFQGRV TITADTSTDATAYLELSSLRSEDATVYYCTGGNYV GWFPYWGQGLTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFLL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGKGGGSGGGGSIWELKKDVYVVELD WYPDAPGEMVVLTCDTPEEDGITWTLTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLL LHKKEDGIWSTDILKDQKEPKNKTFRLCEAKNYS GRFTCWWTITSTDLTFSVKSSRGSSDPQGVTCG AATLSAERVRGDNKEYEYSVEQEDSASPAEES LPIEVMVDAVHKLKYENYTSFFIRDIHKPDPKKNL QKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFSV QVQGKSKREKKDRVFTDKTSATVICRKNASISVR AQDRYYSSSWSEWASVPCS (SEQ ID NO: 169)	humanized M1, p40 C177S
hM1-Fab(H1)- p40	EVQLVQSGAEVKKPGATVKISCTASGFNIKDEYIN WVKQRPKGGLWIGWIDPENGDEYASKFQGRV TITADTSTDATAYLELSSLRSEDATVYYCTGGNYV GWFPYWGQGLTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCGGGSGGGGSIWELKK	humanized M1, p40 C177S

	DVYVVELDWYPDAPGEMVVLTCDTPEEDGITWT LDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGE VLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLR CEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSDP QGVTCGAATLSAERVGRDNKEYEYSVEQCEDSA SPAAEESLPIEVMVDAVHKLKYENYTSFFIRDIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSY FSLTFSVQVQGKSKREKKDRVFTDKTSATVICRK NASISVRAQDRYYSSSWSEWASVPCSGGSGLNDI FEAQKIEWHEGGSHHHHHH (SEQ ID NO: 170)	
hM1-ScFv(vH1- vL4)-p40	EVQLVQSGAEVKKPGATVKISCTASGFNIKDEYIN WVKQRPKGLEWIGWIDPENGDEYASKFQGRV TITADTSTDAYLELSSLRSEDNAVYYCTGGNYV GWFPYWGQGLTVTVSSGGGSGGGGSGGGGSGQI VLTQSPATLSASPGERVMTMSCASSSVSYMHYQ QKPGTSPRRWLYDTSKLASGVPARFSGSGSGTDY TLTISSLEPEDFATYYCQWSSNPPYTFGQGTKLE IKGGGSGGGGSIWELKDVYVVELDWYPDAPG EMVVLTCDTPEEDGITWTLTLDQSSEVLGSGKTLTIQ VKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGI WSTDILKDQKEPKNKTFLRCEAKNYSGRFTCW LTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQCEDSASPAAEESLPIEVMVD AVHKLKYENYTSFFIRDIKPDPPKNLQLKPLKNS RQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKSKR EKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS WSEWASVPCSGGSGLNDIFEAQKIEWHEGGSHH HHHH (SEQ ID NO: 171)	humanized M1, p40 C177S
hM1-ScFv(vL4- vH1)-p40	QIVLTQSPATLSASPGERVMTMSCASSSVSYMHW YQKPGTSPRRWLYDTSKLASGVPARFSGSGSGT DYTLTISSLEPEDFATYYCQWSSNPPYTFGQGTK LEIKGGGSGGGGSGGGGSEVQLVQSGAEVKKP GATVKISCTASGFNIKDEYINWVKQRPKGLEWI GWIDPENGDEYASKFQGRVTITADTSTDAYLE LSSLRSEDNAVYYCTGGNYVGWFPYWGQGLTV VSSGGGSGGGGSIWELKDVYVVELDWYPDAP GEMVVLTCDTPEEDGITWTLTLDQSSEVLGSGKTLTI QVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGI WSTDILKDQKEPKNKTFLRCEAKNYSGRFTCW LTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQCEDSASPAAEESLPIEVMVD AVHKLKYENYTSFFIRDIKPDPPKNLQLKPLKNS RQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKSKR EKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS WSEWASVPCSGGSGLNDIFEAQKIEWHEGGSHH HHHH (SEQ ID NO: 172)	humanized M1, p40 C177S
hM1-Fab(H1)-Fc (knob)	EVQLVQSGAEVKKPGATVKISCTASGFNIKDEYIN WVKQRPKGLEWIGWIDPENGDEYASKFQGRV TITADTSTDAYLELSSLRSEDNAVYYCTGGNYV GWFPYWGQGLTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCGGGSGGGGSEPKSSD KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC	humanized M1, Knob T366W, human IgG1, LALA-PG, effector function null

	KVSNKALGAPIEKTISKAKGQPREPQVCTLPSPRE EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV SCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 173)	
hM1-ScFv(vH1- vL4)-Fc(knob)	EVQLVQSGAEVKKPGATVKISCTASGFNIKDEYIN WVKQRPKGLEWIGWIDPENGDEYASKFQGRV TITADTSTDATAYLELSSLRSEDATVYYCTGGNYV GWFPYWGQGTLLTVSSGGGGSGGGSGGGGSQI VLTQSPATLSASPGERVMTMCSASSSVSYMHYQ QKPGTSPRRWLYDTSKLASGVPARFSGSGSGTDY LTISLLEPEDFATYYCQQWSSNPPYTFGQGTKLE IKGGGGSGGGGSEPKSSDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVCTLPSPREEMTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL YSKLTVDKSRWQQGNVSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO: 174)	humanized M1, Knob T366W, human IgG1, LALA-PG, effector function null
hM1-ScFv(vL4- vH1)-Fc (knob)	QIVLTQSPATLSASPGERVMTMCSASSSVSYMHW YQKPGTSPRRWLYDTSKLASGVPARFSGSGSGT DYTLTISLLEPEDFATYYCQQWSSNPPYTFGQGTK LEIKGGGGSGGGGSEVQLVQSGAEVKKP GATVKISCTASGFNIKDEYINWVKQRPKGLEWI GWIDPENGDEYASKFQGRVTITADTSTDATAYLE LSSLRSEDATVYYCTGGNYVWGFYWGQGTLLTV VSSGGGGSGGGGSEPKSSDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVCTLPSPREEMTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL YSKLTVDKSRWQQGNVSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO: 175)	humanized M1, Knob T366W, human IgG1, LALA-PG, effector function null
p35-hM1- ScFv(vH1-vL4)2	RNLPVATPDPMFPCLLHSHQNLRAVDNMLQKA RQTFEYFCTSEEIDHEDITKDKTSTVEACPLELT KNESKLSNRETSFITNGSCLASRKTSFMMALCLSS IYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNM LAVIDELMQALNFNSETVPQKSSLEEDFYKTKIK LCILLHAFRIRAVTINRVMSYLNASGGGGSGGGG SEVQLVQSGAEVKKPGATVKISCTASGFNIKDEYI NWVKQRPKGLEWIGWIDPENGDEYASKFQGR VTITADTSTDATAYLELSSLRSEDATVYYCTGGNY VGWFPYWGQGTLLTVSSGGGGSGGGSGGGGS QIVLTQSPATLSASPGERVMTMCSASSSVSYMHW YQKPGTSPRRWLYDTSKLASGVPARFSGSGSGT DYTLTISLLEPEDFATYYCQQWSSNPPYTFGQGTK LEIKGGSGGGSGGGSGGGSGGGSGGGSGGGE VQLVQSGAEVKKPGATVKISCTASGFNIKDEYIN WVKQRPKGLEWIGWIDPENGDEYASKFQGRV TITADTSTDATAYLELSSLRSEDATVYYCTGGNYV GWFPYWGQGTLLTVSSGGGGSGGGSGGGGSQI VLTQSPATLSASPGERVMTMCSASSSVSYMHYQ QKPGTSPRRWLYDTSKLASGVPARFSGSGSGTDY LTISLLEPEDFATYYCQQWSSNPPYTFGQGTKLE	humanized M1, p35 S27D, C74K, D188N

	IKGGSGLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 176)	
p35-hM1-ScFv(vL4-vH1)2	RNLPVATPDPGMFPCLLHHSQNLLRAVDNMLQKARQTLEFYPCCTSEEDHEDITKDKTSTVEACLPLELTKNESKLSNRETSFITNGSCLASRKTSFMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNM LAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTINRVMSYLNASGGGGSGGGGSQIVLTQSPATLSASPGERVMTSCSASSSVSYMH WYQQKPGTSPRRWLYDTSKLASGVPARFSGSGSGTDYTLTISSLEPEDFATYYCQQWSSNPPYTFGQGTKLEIKGGGGSGGGSGGGGSEVQLVQSGAEVKKPGATVKISCTASGFNIKDEYINWVKQRPKGLEWIGWIDPENGDEYASKFQGRVTITADTSTDYALELSSLRSEDYAVYYCTGGNYVGWFPYWGQGLVTVSSGGSGGGSGGGSGGGSGGGSGGGSGGQIVLTQSPATLSASPGERVMTSCSASSSVSYMHWYQQKPGTSPRRWLYDTSKLASGVPARFSGSGSGTDYTLTISSLEPEDFATYYCQQWSSNPPYTFGQGTKLEIKGGGGSGGGSGGGGSEVQLVQSGAEVKKPGATVKISCTASGFNIKDEYINWVKQRPKGLEWIGWIDPENGDEYASKFQGRVTITADTSTDYALELSSLRSEDYAVYYCTGGNYVGWFPYWGQGLVTVSSGGSLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 177)	humanized M1, p35 S27D, C74K, D188N
hM1-IgG1(H3)-p40	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYINWVKQRPQGQLEWIGWIDPENGDEYASKFQGRATITADTSTDYALELSSLRSEDYAVYYCTGGNYVGWFPYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSIWELKKDYYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVKEFGDAGQYTKHGGEVLSHSLLL LHKKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRGNKEYEYSVEQCEDSASPAAEESLPIEVMVDAVHKLKYENYTSFFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS (SEQ ID NO: 178)	humanized M1, p40 C177S
hM1-Fab(H3)-p40	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYINWVKQRPQGQLEWIGWIDPENGDEYASKFQGRATITADTSTDYALELSSLRSEDYAVYYCTGGNYVGWFPYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGGGGSGGGGSIWELK KDYYVVELDWYPDAPGEMVVLTCDTPEEDGITW	humanized M1, p40 C177S

	TLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGG EVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTF RCEAKNYSGRFTCWWLTISTDLTFSVKSSRGSS DPQGVTCGAATLSAERVRGDNKEYEYSVEQED SASPAAEESLPIEVMVDAVHKLKYENYTSSFFIR IIPDPKPNLQKPLKNSRQVEVSWEYPDTWSTPH SYFSLTFSVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGGSGLN DIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 179)	
hM1-ScFv(vH3-vL1)-p40	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYI NWVKQRPQGQLEWIGWIDPENGDEYASKFQGR ATITADTSTDYALELSSLRSEDYAVYYCTGGNY VGWFPYWGQGTLLTVSSGGGGSGGGSGGGGS EIVLTQSPATLSLSPGERATLSCSASSSVSYMHY QQKPGTSPRRWIYDTSKLASGVPARFSGSGSGTD FTLTISLLEPEDFAVYYCQQWSSNPPYTFGQGT EIKGGGGSGGGGSIWELKKDVYVVELDWYDPAP GEMVVLTCDTPEEDGITWTLTLDQSSEVLGSGKTLTI QVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGI WSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWW LTISTDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQEDSASPAAEESLPIEVMVD AVHKLKYENYTSSFFIRDIIPDPKPNLQKPLKNS RQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKSKR EKKDRVFTDKTSATVICRKNASISVRAQDRYYSS WSEWASVPCSGGSGLNDIFEAQKIEWHEGGSHH HHHH (SEQ ID NO: 180)	humanized M1, p40 C177S
hM1-ScFv(vL1-vH3)-p40	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHY QQKPGTSPRRWIYDTSKLASGVPARFSGSGSGTD FTLTISLLEPEDFAVYYCQQWSSNPPYTFGQGT EIKGGGGSGGGGSGGGGSEVQLQQSGAEVKKPG ATVKLSCTASGFNIKDEYNWVKQRPQGQLEWIG WIDPENGDEYASKFQGRATITADTSTDYALELS SLRSEDYAVYYCTGGNYVGWFPYWGQGTLLTVS SGGGSGGGGSIWELKKDVYVVELDWYDPAPGE MVVLTCDTPEEDGITWTLTLDQSSEVLGSGKTLTIQ VKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGI WSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWW LTISTDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVEQEDSASPAAEESLPIEVMVD AVHKLKYENYTSSFFIRDIIPDPKPNLQKPLKNS RQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKSKR EKKDRVFTDKTSATVICRKNASISVRAQDRYYSS WSEWASVPCSGGSGLNDIFEAQKIEWHEGGSHH HHHH (SEQ ID NO: 181)	humanized M1, p40 C177S
hM1-Fab(H3)-Fc (knob)	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYI NWVKQRPQGQLEWIGWIDPENGDEYASKFQGR ATITADTSTDYALELSSLRSEDYAVYYCTGGNY VGWFPYWGQGTLLTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTQYICNV NHKPSNTKVDKKVEPKSCGGGGSGGGGSEPKSS DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALGAPIEKISKAKGQPREPQVCTLPSSR	humanized M1, Knob T366W, human IgG1, LALA-PG, effector function null

	EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPE NNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 182)	
hM1-ScFv(vL1- vH3)-Fc (knob)	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWY QQKPGTSPRRWIYDTSKLASGVPARFSGSGSGTD FTLTISLLEPEDFAVYYCQQWSSNPPYTFGQGTKL EIKGGGSGGGGSGGGGSEVQLQQSGAEVKKPG ATVKLSCTASGFNIKDEYINWVKQRPQGQLEWIG WIDPENGDTHEYASKFQGRATITADTSTDYALELS SLRSEDYAVYYCTGGNYVGWFPYWGQGLTVTS SGGGSGGGGSGGGGSEPKSSDKTHTCPPCPAEEAAGGP SVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVCTLPPSREEMTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO: 183)	humanized M1, Knob T366W, human IgG1, LALA-PG, effector function null
p35-hM1- ScFv(vL1-vH3)2	RNLPVATPDGPMFPCLLHHSQNLLRAVDNMLQKA RQTLEFYPTSEEIDHEDITDKDTSTVEACLPLELT KNESKLNRETSFITNGSCLASRKTSFMMALCLSS IYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNM LAVIDELMQALNFNSETVPQKSSLEEDFYKTKIK LCILLHAFRIRAVTINRVMSYLNASGGGSGGGG SEIVLTQSPATLSLSPGERATLSCSASSSVSYMHW YQQKPGTSPRRWIYDTSKLASGVPARFSGSGSGT DFTLTISLLEPEDFAVYYCQQWSSNPPYTFGQGTK LEIKGGGSGGGGSGGGGSEVQLQQSGAEVKKP GATVKLSCTASGFNIKDEYINWVKQRPQGQLEWI GIDPENGDTHEYASKFQGRATITADTSTDYALE LSSLRSEDYAVYYCTGGNYVGWFPYWGQGLTVT VSSGGSGGGGSGGGGSGGGGSGGGGSGGGSGGEI VLTQSPATLSLSPGERATLSCSASSSVSYMHWYQ QKPGTSPRRWIYDTSKLASGVPARFSGSGSGTDFT LTISLLEPEDFAVYYCQQWSSNPPYTFGQGTKLEI KGGGSGGGGSGGGGSEVQLQQSGAEVKKPGAT VKLSCTASGFNIKDEYINWVKQRPQGQLEWIGWI DPENGDTHEYASKFQGRATITADTSTDYALELSSL RSEDYAVYYCTGGNYVGWFPYWGQGLTVTVSS GGGSLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 184)	humanized M1, p35 S27D, C74K, D188N
hM1-ScFv(vH3- vL4)-p40	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYI NWVKQRPQGQLEWIGWIDPENGDTHEYASKFQGR ATITADTSTDYALELSSLRSEDYAVYYCTGGNY VGWFPYWGQGLTVTVSSGGGSGGGGSGGGGS QIVLTQSPATLSASPERVTMCSASSSVSYMHW YQQKPGTSPRRWLVDTSKLASGVPARFSGSGSGT DYTLTISLLEPEDFATYYCQQWSSNPPYTFGQGTK LEIKGGGSGGGGSIWELKKDVYVVELDWYPDA PGEMVVLTCDTPEEDGITWTLDSSEVLGSGKTL TIQVKEFGDAGQYTCHKGGEVLSHSLLLHKKED GIWSTDILKDKQEPKNTFLRCEAKNYSGRFTCW WLTITISDLTFSVKSSRGSSDPQGVTCGAATLSAE RVRGDNKEYEYSVEQCEDSASPAAEESLPIEVMV DAVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLK	humanized M1, p40 C177S

	NSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKS KREKKDRVFTDKTSATVICRKNASISVRAQDRYY SSSWSEWASVPCSGGSGGLNDIFEAQKIEWHEGGG HHHHHH (SEQ ID NO: 185)	
hM1-ScFv(vL4- vH3)-p40	QIVLTQSPATLSASPGERVMTMCSASSSVSYMHW YQOKPGTSPRRWLYDTSKLASGVPARFSGSGSGT DYTLTISSLEPEDFATYYCQQWSSNPPYTFGQGTK LEIKGGGGSGGGGSGGGGSEVQLQQSGAEVKKP GATVKLSCTASGFNIKDEYINWVKQRPQGGLWEI GWIDPENGDEYASKFQGRATITADTSTDATYLE LSSLRSEDATVYYCTGGNYVGWFPYWGQGLVT VSSGGGGSGGGGSIWELKKDVYVVELDWYPDAP GEMVVLTCDTPEEDGITWTLDSSEVLGSGKTLTI QVKEFGDAGQYTCHKGGEVLSHSLLLHKKEDGI WSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWW LTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVECQEDSASPAAEESLPIEVMVD AVHKLKYENYTSFFIRDIIKPDPPKNLQLKPLKNS RQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKSKR EKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS WSEWASVPCSGGSGGLNDIFEAQKIEWHEGGSHH HHHH (SEQ ID NO: 186)	humanized M1, p40 C177S
hM1-ScFv(vH3- vL4)-Fc(knob)	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYI NWVKQRPQGGLWEIGWIDPENGDEYASKFQGR ATITADTSTDATYLELSSLRSEDATVYYCTGGNY VGWFPYWGQGLVTVSSGGGGSGGGGSGGGGS QIVLTQSPATLSASPGERVMTMCSASSSVSYMHW YQOKPGTSPRRWLYDTSKLASGVPARFSGSGSGT DYTLTISSLEPEDFATYYCQQWSSNPPYTFGQGTK LEIKGGGGSGGGGSEPKSSDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVCTLPSPREEMTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPVLDSDGSFLL YSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQ KSLSLSPGK (SEQ ID NO: 187)	humanized M1, Knob T366W, human IgG1, LALA-PG, effector function null
hM1-ScFv(vL4- vH3)-Fc (knob)	QIVLTQSPATLSASPGERVMTMCSASSSVSYMHW YQOKPGTSPRRWLYDTSKLASGVPARFSGSGSGT DYTLTISSLEPEDFATYYCQQWSSNPPYTFGQGTK LEIKGGGGSGGGGSGGGGSEVQLQQSGAEVKKP GATVKLSCTASGFNIKDEYINWVKQRPQGGLWEI GWIDPENGDEYASKFQGRATITADTSTDATYLE LSSLRSEDATVYYCTGGNYVGWFPYWGQGLVT VSSGGGGSGGGGSEPKSSDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVCTLPSPREEMTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPVLDSDGSFLL YSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQ KSLSLSPGK (SEQ ID NO: 188)	humanized M1, Knob T366W, human IgG1, LALA-PG, effector function null
p35-hM1- ScFv(vH3-vL4)2	RNLPVATPDPMFPCLLHHSQNLLRAVDNMLQKA RQTLEFYPTSEEIDHEDITDKTSTVEACLPLELT KNESKLNRETSFITNGSCLASRKTSFMMALCLSS IYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNM	humanized M1, p35 S27D, C74K, D188N

	LAVIDELMQALNFNSETVPQKSSLEEDFYKTKIK LCILLHAFRIRAVTINRVMSYLNASGGGGSGGGG SEVQLQQSGAEVKKPGATVKLSCTASGFNIKDEY INWVKQRPQGQLEWIGWIDPENGDEYASKFQG RATITADTSTDYAYLELSSLRSEDYAVYYCTGGN YVGWFPYWGQGLTVTVSSGGGGSGGGSGGGG SQIVLTQSPATLSASPGERVMTSCSASSSVSYMH WYQQKPGTSPRRWLYDTSKLASGVPARFSGSGS GTDYTLTISSLEPEDFATYYCQQWSSNPPYTFGQG TKLEIKGGSGGGSGGGSGGGSGGGSGGGSGG GEVQLQQSGAEVKKPGATVKLSCTASGFNIKDEY INWVKQRPQGQLEWIGWIDPENGDEYASKFQG RATITADTSTDYAYLELSSLRSEDYAVYYCTGGN YVGWFPYWGQGLTVTVSSGGGGSGGGSGGGG SQIVLTQSPATLSASPGERVMTSCSASSSVSYMH WYQQKPGTSPRRWLYDTSKLASGVPARFSGSGS GTDYTLTISSLEPEDFATYYCQQWSSNPPYTFGQG TKLEIKGGSGLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 189)	
p35-hM1- ScFv(vL4-vH3)2	RNLPVATPDPMFPCPLHHSQNLLRAVDNMLQKA RQTLEFYPTSEEIDHEDITKDKTSTVEACLPLELT KNESKLNSRETSFITNGSCLASRKTSFMMALCLSS IYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNM LAVIDELMQALNFNSETVPQKSSLEEDFYKTKIK LCILLHAFRIRAVTINRVMSYLNASGGGGSGGGG SQIVLTQSPATLSASPGERVMTSCSASSSVSYMH WYQQKPGTSPRRWLYDTSKLASGVPARFSGSGS GTDYTLTISSLEPEDFATYYCQQWSSNPPYTFGQG TKLEIKGGGGSGGGSGGGSGGGSGGGSGGGSGG KPGATVKLSCTASGFNIKDEYINWVKQRPQGQLE WIGWIDPENGDEYASKFQGRATITADTSTDYAY LELSSLRSEDYAVYYCTGGNYVGWFPYWGQGLT VTVSSGGSGGGSGGGSGGGSGGGSGGGSGGGSGG QIVLTQSPATLSASPGERVMTSCSASSSVSYMHW YQQKPGTSPRRWLYDTSKLASGVPARFSGSGSGT DYTLTISSLEPEDFATYYCQQWSSNPPYTFGQGTK LEIKGGGGSGGGSGGGSGGGSGGGSGGGSGGGG GATVKLSCTASGFNIKDEYINWVKQRPQGQLEWI GWIDPENGDEYASKFQGRATITADTSTDYAYLE LSSLRSEDYAVYYCTGGNYVGWFPYWGQGLTVT VSSGGSGLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 190)	humanized M1, p35 S27D, C74K, D188N
P35-M1-ScFv (H3L1)2	RNLPVATPDPMFPCPLHHSQNLLRAVDNMLQKA RQTLEFYPTSEEIDHEDITKDKTSTVEACLPLELT KNESKLNSRETSFITNGSCLASRKTSFMMALCLSS IYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNM LAVIDELMQALNFNSETVPQKSSLEEDFYKTKIK LCILLHAFRIRAVTINRVMSYLNASGGGGSGGGG SEVQLQQSGAEVKKPGATVKLSCTASGFNIKDEY INWVKQRPQGQLEWIGWIDPENGDEYASKFQG RATITADTSTDYAYLELSSLRSEDYAVYYCTGGN YVGWFPYWGQGLTVTVSSGGGGSGGGSGGGG SEIVLTQSPATLSLSPGERATLSCSASSSVSYMHW YQQKPGTSPRRWLYDTSKLASGVPARFSGSGSGT DFTLTISSLEPEDFATYYCQQWSSNPPYTFGQGTK LEIKGGSGGGSGGGSGGGSGGGSGGGSGGGSGGE	humanized M1, p35 S27D, C74K, D188N

	VQLQQSGAEVKKPGATVKLSCTASGFNIKDEYIN WVKQRPQGQLEWIGWIDPENGDEYASKFQGRA TITADTSTDYAYLELSSLRSEDYAVYYCTGGNYV GWFPYWGQGTLLTVSSGGGSGGGGSGGGGSEI VLTQSPATLSLSPGERATLSCSASSSVSYMHWYQ QKPGTSPRRWIYDTSKLASGVPARFSGSGSGTDFT LTISLEPEDFAVYYCQQWSSNPPYTFGQGTKLEI KGGSGLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 191)	
M1-ScFv (H3L1)- Fc (knob)	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYI NWVKQRPQGQLEWIGWIDPENGDEYASKFQGR ATITADTSTDYAYLELSSLRSEDYAVYYCTGGNY VGWFPYWGQGTLLTVSSGGGSGGGGSGGGGSEI VLTQSPATLSLSPGERATLSCSASSSVSYMHWY QKPGTSPRRWIYDTSKLASGVPARFSGSGSGTD FTLTISLEPEDFAVYYCQQWSSNPPYTFGQGTKL EIKGGGSGGGGSEPKSSDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYCKVSNKALGAPIEKTISK AKGQPREPQVCTLPSPREEMTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPVLDSDGSFLL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO: 192)	humanized M1, Knob T366W, human IgG1, LALA-PG, effector function null

Table 29 – Humanized M8 antibody-IL-12 subunit fusion amino acid sequences

Description	Sequence	Notes
hM8-IgG1(H3)- p40	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPVLDSDGSFLLYSKLTVD DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGSGGGGSIWELKKDVYVVELDWYPDAP GEMVVLTCDTPEEDGITWTLDSSEVLGSGKTLTI QVKEFGDAGQYTCHKGGEVLSHSLLLHKKEDGI WSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWW LTTISTDLTFSVKSRRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVECEQEDSASPAEEESLPIEVMVD AVHKLKYENYTSFFIRDIIPDPKPNLQLKPLKNS RQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKSKR EKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS WSEWASVPCS (SEQ ID NO: 193)	humanized M8 LALA, p40 C177S
hM8-Fab(H3)- p40	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGT	humanized M8, p40 C177S

	AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGGGGSGGGGSIWELKKDVYVV ELDWYPDAPGEMVVLTCDTPEEDGITWTLDSSE VLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSL LLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKN YSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTC GAATLSAERVRGDNKEYEYSVEQEDSASPAAEE SLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFS VQVQGKSKREKKDRVFTDKTSATVICRKNASISV RAQDRYYSSSWSEWASVPCSGGSLNDIFEAQKI EWHEGGSHHHHHH (SEQ ID NO: 194)	
hM8-Fab(h3)-Fc (Knob)	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGGGGSGGGGSEPKSSDKTHTCP PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL GAPIEKTISKAKGQPREPQVCTLPSSREEMTKNQV SLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPGK (SEQ ID NO: 195)	humanized M8, Knob T366W, human IgG1, LALA-PG, effector function null
hM8-ScFv(vH3- vL2B2)-p40	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSGGGGSGGGGSGGGGSDIQMT QSPSSLSASVGDTVTITCRTSENIYSNLAWYQQK GKSPQLLVYAATNLADGVPSRFSGSGSGTQYTLK ISSLPEDFATYYCQHFHWGTPWTFGGGKVEIKG GGGSGGGGSIWELKKDVYVVELDWYPDAPGEM VVLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVK EFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWST DILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTI STDLTFSVKSSRGSSDPQGVTCGAATLSAERVRG DNKEYEYSVEQEDSASPAAEESLPIEVMVDAVH KLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQV EVSWEYPDTWSTPHSYFSLTFSVQVQGKSKREKK DRVFTDKTSATVICRKNASISVRAQDRYYSSSWSE WASVPCSGGSLNDIFEAQKIEWHEGGSHHHHHH H (SEQ ID NO: 196)	humanized M8, p40 C177S
hM8- ScFv(vL2B2- vH3)-p40	DIQMTQSPSSLSASVGDTVTITCRTSENIYSNLAW YQQKPGKSPQLLVYAATNLADGVPSRFSGSGSGT QYTLKISSLPEDFATYYCQHFHWGTPWTFGGGK VEIKGGGGSGGGGSGGGGSGIQLVQSGSELKKPG ASVKISCKASGTTFTDYGMNWVKQSPGKGLK MGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYL QISSLKAEDTATYFCAHYSFDYWGQGTLLTVSSG GGGSGGGGSIWELKKDVYVVELDWYPDAPGEM VVLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVK EFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWST DILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTI	humanized M8, p40 C177S

	STDLTFSVKSSRGSSDPQGVTCGAATLSAERVGR DNKEYEYSVEQEDSASPAAEESLPIEVMVDAVH KLKYENYTSFFIRDIIKPDPPKLNQLKPLKNSRQV EVSWEYPDTWSTPHSYFSLTFSVQVQGKSKREKK DRVFTDKTSATVICRKNASISVRAQDRYYSSSWSE WASVPCSGGSLNDIFEAQKIEWHEGGSHHHHH H (SEQ ID NO: 197)	
hM8-ScFv(vH3- vL2B2)-Fc (knob)	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSGGGGSGGGSGGGGSDIOMT QSPSSLSASVGDVTITCRTSENIYSNLAWYQQKP GKSPQLLVYAATNLADGVPSRFSGSGSGTQYTLK ISSLPEDFATYYCQHFVGTPWTFGGGKVEIKG GGGSGGGGSEPKSSDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALGAPIEKTISKAKG QPREPQVCTLPPSREEMTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS LSPGK (SEQ ID NO: 198)	humanized M8, Knob T366W, human IgG1, LALA-PG, effector function null
hM8- ScFv(vL2B2- vH3)-Fc (knob)	DIOMTQSPSSLSASVGDVTITCRTSENIYSNLAW YQQKPGKSPQLLVYAATNLADGVPSRFSGSGSGT QYTLKISSLPEDFATYYCQHFVGTPWTFGGGK VEIKGGGGSGGGSGGGSGGGGSIQLVQSGSELKKPG ASVKISCKASGTTFTDYGMNWVKQSPGKGLKW MGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYL QISSLKAEDTATYFCAHYSFDYWGQGTLLTVSSG GGGSGGGGSEPKSSDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALGAPIEKTISKAKG QPREPQVCTLPPSREEMTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS LSPGK (SEQ ID NO: 199)	humanized M8, Knob T366W, human IgG1, LALA-PG, effector function null
p35-hM8- ScFv(vH3- vL2B2)	RNLPVATPDGPMFPCLLHHSQNLLRAVDNMLQKA RQTLEFYPTSEEIDHEDITKDKTSTVEACLPLELT KNESKLNSRETSFITNGSCLASRKTSFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNML AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKL CILLHAFRIRAVTINRVMSYLNASGGGGSGGGGS QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSGGGGSGGGSGGGGSDIOMT QSPSSLSASVGDVTITCRTSENIYSNLAWYQQKP GKSPQLLVYAATNLADGVPSRFSGSGSGTQYTLK ISSLPEDFATYYCQHFVGTPWTFGGGKVEIKG GSGGGSGGGSGGGSGGGSGGGSGGGGSIQLVQ SGSELKKPGASVKISCKASGTTFTDYGMNWVKQS PGKGLKWMGWINTNTGEPTYAEDFKGRFVFSLD TSVSTAYLQISSLKAEDTATYFCAHYSFDYWGQ TLLTVSSGGGGSGGGSGGGGSDIOMTQSPSSLS ASVGDVTITCRTSENIYSNLAWYQQKPGKSPQL	humanized M8, p35 S27D, C74K, D188N

	LVYAATNLADGVPSRFSGSGSGTQYTLKISSLQPE DFATYYCQHFHWGTPWTFGGGKVEIKGGGSLNDI FEAQKIEWHEGGSHHHHHH (SEQ ID NO: 200)	
p35-hM8- ScFv(vL2B2- vH3)	RNLPVATPDPGMFPCLLHHSQNLLRAVDNMLQKA RQTLEFYPTSEEIDHEDITKDKTSTVEACLPLELT KNESKLNSRETSFITNGSCLASRKTSFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNML AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKL CILLHAFRIRAVTINRVMSYLNASGGGGSGGGGS DIQMTQSPSSLSASVGDVTITCRTSENIYSNLAW YQKPGKSPQLLVYAATNLADGVPSRFSGSGSGT QYTLKISSLQPEDFATYYCQHFHWGTPWTFGGGK VEIKGGGGSGGGSGGGGSIQLVQSGSELKKPG ASVKISCKASGTTFTDYGMNWVKQSPGKGLKW MGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYL QISSLKAEDTATYFCAHYSFDYWGQGTLLTVSSG SGGGGGSGGGSGGGSGGGSGGGSGGGDIQMTQ SPSSLSASVGDVTITCRTSENIYSNLAWYQKPG KSPQLLVYAATNLADGVPSRFSGSGSGTQYTLKIS SLQPEDFATYYCQHFHWGTPWTFGGGKVEIKGG GGGGGGSGGGSGGGGSIQLVQSGSELKKPGASVKIS CKASGTTFTDYGMNWVKQSPGKGLKWMGWINT NTGEPTYAEDFKGRFVFSLDTSVSTAYLQISSLKA EDTATYFCAHYSFDYWGQGTLLTVSSGGGSLNDI FEAQKIEWHEGGSHHHHHH (SEQ ID NO: 201)	humanized M8, p35 S27D, C74K, D188N
hM8-ScFv(vH3- vL2B5)-p40	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSGGGGSGGGSGGGGSDIQMT QSPSSLSASVGDVTITCRTSENIYSNLAWYQKPG KSPQLLVYAATNLADGVPSRFSGSGSGTQYTLTI SSLQPEDFGTYCQHFHWGTPWTFGGGKVEIKGG GGGGGGSIWELKKDVYVVELDWYDPDAPGEMV VLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVKE FGDAGQYTCHKGGEVLSHSLLLHKKEDGIWSTD ILKDQKEPKNKTFRLCEAKNYSGRFTCWWLTTIS TDLTFSVKSSRGSSDPQGVTCGAATLSAERVRG NKEYEYSVEQCEDSASPAAEESLPIEVMVDAVHK LKYENYTSSFFIRDIKPDPPKNLQLKPLKNSRQVE VSWEYPDTWSTPHSYFSLTFSVQVQGKSKREKKD RVFTDKTSATVICRKNASISVRAQDRYSSSWSE WASVPCSGGSLNDIFEAQKIEWHEGGSHHHHHH H (SEQ ID NO: 202)	humanized M8, p40 C177S
hM8- ScFv(vL2B5- vH3)-p40	DIQMTQSPSSLSASVGDVTITCRTSENIYSNLAW YQKPGKSPQLLVYAATNLADGVPSRFSGSGSGT DYTLTISSLQPEDFGTYCQHFHWGTPWTFGGGK VEIKGGGGSGGGSGGGGSIQLVQSGSELKKPG ASVKISCKASGTTFTDYGMNWVKQSPGKGLKW MGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYL QISSLKAEDTATYFCAHYSFDYWGQGTLLTVSSG GGGGGGSIWELKKDVYVVELDWYDPDAPGEM VLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVK EFGDAGQYTCHKGGEVLSHSLLLHKKEDGIWST DILKDQKEPKNKTFRLCEAKNYSGRFTCWWLTTI STDLTFSVKSSRGSSDPQGVTCGAATLSAERVRG DNKEYEYSVEQCEDSASPAAEESLPIEVMVDAVH	humanized M8, p40 C177S

	KLKYENYTSFFIRDIIKPDPPKNLQLKPLKNSRQV EVSWEYPDTWSTPHSYFSLTFSVQVQGKSKREKK DRVFTDKTSATVICRKNASISVRAQDRYYSSSWSE WASVPCSGGSGLNDIFEAQKIEWHEGGSHHHHH H (SEQ ID NO: 203)	
hM8-ScFv(vH3- vL2B5)-Fc (knob)	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSGGGGSGGGSGGGGSDIQMT QSPSSLASVGDRVTITCRTSENIYSNLAWYQKP GKSPQLLVY AATNLADGVPSRFSGSGSGTDYTLTI SSLQPEDFGTYCQHFWGTPWTFGGGKVEIKGG GGSGGGGSEPKSSDKTHTCPPCPAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPSPREEMTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLS PGK (SEQ ID NO: 204)	humanized M8, Knob T366W, human IgG1, LALA-PG, effector function null
hM8- ScFv(vL2B5- vH3)-Fc (knob)	DIQMTQSPSSLSASVGDRVTITCRTSENIYSNLAW YQKPKGKSPQLLVY AATNLADGVPSRFSGSGSGT DYTLTISSLQPEDFGTYCQHFWGTPWTFGGGK VEIKGGGGSGGGSGGGSGGGGSIQLVQSGSELKKPG ASVKISCKASGTTFTDYGMNWVKQSPGKGLK WMGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYL QISSLKAEDTATYFCAHYSFDYWGQGTLLTVSSG GGGGGGGSEPKSSDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALGAPIEKTISKAKG QPREPQVCTLPSPREEMTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS LSPGK (SEQ ID NO: 205)	humanized M8, Knob T366W, human IgG1, LALA-PG, effector function null
p35-hM8- ScFv(vH3- vL2B5)	RNLPVATPDGPMFPCLLHHSQNLLRAVDNMLQKA RQTLEFYPTSEEIDHEDITKDKTSTVEACLPLELT KNESKLNSRETSFITNGSCLASRKTSFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNML AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKL CILLHAFRIRAVTINRVMSYLNASGGGGSGGGGS QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSGGGGSGGGSGGGGSDIQMT QSPSSLASVGDRVTITCRTSENIYSNLAWYQKP GKSPQLLVY AATNLADGVPSRFSGSGSGTDYTLTI SSLQPEDFGTYCQHFWGTPWTFGGGKVEIKGG SGGGGGSGGGSGGGSGGGSGGGGSIQLVQS GSELKKPGASVKISCKASGTTFTDYGMNWVKQSP GKGLKWMGWINTNTGEPTYAEDFKGRFVFSLD SVSTAYLQISSLKAEDTATYFCAHYSFDYWGQGT LLTVSSGGGGSGGGSGGGGSDIQMTQSPSSLSAS VGDRVTITCRTSENIYSNLAWYQKPKGKSPQLLV YAATNLADGVPSRFSGSGSGTDYTLTISSLQPEDF GTYYCQHFWGTPWTFGGGKVEIKGGSGLNDIFE	6humanized M8, p35 S27D, C74K, D188N

[illegible]

	EVSWEYPDTWSTPHSYFSLTFSVQVQGKSKREKK DRVFTDKTSATVICRKNASISVRAQDRYYSSSWSE WASVPCSGGSLNDIFEAQKIEWHEGGSHHHHH H (SEQ ID NO: 209)	
hM8-ScFv(vH3- vL2B10)-Fc (knob)	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSGGGGSGGGSGGGGSDIQMT QSPSSLSASVGDVTITCRTSENIYSNLAWYQQKP GKSPQLLVY AATNLADGVPSRFSGSGSGTDYTLTI SSLQPEDFGTYQCQHFWGTPWTFGGGKVEIKGG GGSGGGGSEPKSSDKTHTCPPCPAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPPSREEMTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK (SEQ ID NO: 210)	humanized M8, Knob T366W, human IgG1, LALA-PG, effector function null
hM8- ScFv(vL2B10- vH3)-Fc (knob)	DIQMTQSPSSLSASVGDVTITCRTSENIYSNLAW YQQKPGKSPQLLVY AATNLADGVPSRFSGSGSGT DYTLTISSLQPEDFGTYQCQHFWGTPWTFGGGK VEIKGGGGSGGGSGGGSGGGGSIQILVQSGSELKKPG ASVKISCKASGTTFTDYGMNWVKQSPGKGLK MGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYL QISSLKAEDTATYFCAHYSFDYWGQGTLLTVSSG GGSGGGGSEPKSSDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYCKVSNKALGAPIEKTISKAKG QPREPQVCTLPPSREEMTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGK (SEQ ID NO: 211)	humanized M8, Knob T366W, human IgG1, LALA-PG, effector function null
p35-hM8- ScFv(vH3- vL2B10)	RNLPVATPDPMFPCLLHHSQNLRAVDNMLQKA RQTLEFYPTSEEIDHEDITKDKTSTVEACLPLELT KNESKLNSRETSFITNGSCLSRKTSFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNML AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKL CILLHAFRIRAVTINRVMSYLNASGGGGSGGGGS QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFSLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSGGGGSGGGSGGGGSDIQMT QSPSSLSASVGDVTITCRTSENIYSNLAWYQQKP GKSPQLLVY AATNLADGVPSRFSGSGSGTDYTLTI SSLQPEDFGTYQCQHFWGTPWTFGGGKVEIKGG SGGGSGGGSGGGSGGGSGGGSGGGQIQLVQS GSELKKPGASVKISCKASGTTFTDYGMNWVKQSP GKGLKWMGWINTNTGEPTYAEDFKGRFVSLDT SVSTAYLQISSLKAEDTATYFCAHYSFDYWGQGT LLTVSSGGGGSGGGSGGGGSDIQMTQSPSSLSAS VGDVTITCRTSENIYSNLAWYQQKPGKSPQLLV YAATNLADGVPSRFSGSGSGTDYTLTISSLQPEDF GTYQCQHFWGTPWTFGGGKVEIKGGSGLNDIFE AQKIEWHEGGSHHHHHH (SEQ ID NO: 212)	humanized M8, p35 S27D, C74K, D188N

p35-hM8- ScFv(vL2B10- vH3)	RNLPVATPDPMFPCLLHHSQNLLRAVDNMLQKA RQTLEFYPTCTSEEIDHEDITKDKTSTVEACLPLELT KNESKLNSRETSFITNGSCLASRKTSFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNML AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKL CILLHAFRIRAVTINRVMSYLNASGGGGSGGGGS DIQMTQSPSSLSASVGDVTITCRTSENIYSNLAW YQQKPGKSPQLLVYAATNLADGVPSRFSGSGSGT DYTLTISSLQPEDFGTYCQHFHWGTPWTFGGGK VEIKGGGGSGGGSGGGSGGGSGGIQLVQSGSELKKPG ASVKISCKASGTTFTDYGMNWVKQSPGKGLKW MGWINTNTGEPTYAEDFKGRFVFLDTSVSTAYL QISSLKAEDTATYFCAHYSFDYWGQGTLLTVSSG SGGGGGSGGGSGGGSGGGSGGGSGGGSGGDIQMTQ SPSSLSASVGDVTITCRTSENIYSNLAWYQQKPG KSPQLLVYAATNLADGVPSRFSGSGSGTDYTLTIS SLQPEDFGTYCQHFHWGTPWTFGGGKVEIKGG GGSGGGSGGGSGGGSGGGSGGGSGGGSGGASVKIS CKASGTTFTDYGMNWVKQSPGKGLKWMGWINT NTGEPTYAEDFKGRFVFLDTSVSTAYLQISSLKA EDTATYFCAHYSFDYWGQGTLLTVSSGSGGLNDI FEAQKIEWHEGGSHHHHHH (SEQ ID NO: 213)	humanized M8, p35 S27D, C74K, D188N
hM8-IgG1(H3)- scL12	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEAAGGSPVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGSGGGSGGGSGGGSGGGSGGWDYDAP GEMVVLTCDTPEEDGITWTLDSSEVLGSGKTLTI QVKEFGDAGQYTCHKGGEVLSHSLLLHKKEDGI WSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWW LTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAER VRGDNKEYEYSVECEQEDSACPAAEESLPIEVMVD AVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLKNS RQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKR EKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS WSEWASVPCSGGGSGGGSGGGSGGRNLPVATPDPMF PCLHHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEI DHEDITKDKTSTVEACLPLELTKNESCLNSRETSFI TNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFK TMNAKLLMDPKRQIFLDQNMLAVIDELMQALNF NSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVT IDRVMSYLNAS (SEQ ID NO: 214)	humanized M8 LALA, p40-p35
hM8-Fab(H3)- scL12	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGM NWVKQSPGKGLKWMGWINTNTGEPTYAEDFKG RFVFLDTSVSTAYLQISSLKAEDTATYFCAHYSF DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV	humanized M8, p40- p35

	LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGGGGSGGGGSIWELKKDVYVV ELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSE VLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSL LLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKN YSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTC GAATLSAERVRGDNKEYEYSVEQCEDSACPAAEE SLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFC VQVQGKSKREKKDRVFTDKTSATVICRKNASISV RAQDRYYSSSWSEWASVPCSGGGSGGGSGGRNL PVATPDPMFPCLLHHSQNLRLRAVSNMLQKARQT LEFYPCCTSEEIDHEDITKDKTSTVEACLPLELTKNE SCLNSRETSFITNGSCLASRKTSFMMALCLSSIYED LKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVI DELMQALNFNSETVPQKSSLEEPDFYKTKIKLCIL LHAFRIRAVTIDRVMSYLNASGGSGGHHHHHHG GSLNDIFEAQKIEWHE (SEQ ID NO: 215)	
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Table 30 – Humanized M43 antibody-IL-12 subunit fusion amino acid sequences

No	Description	Sequence	Notes
1	hM43-IgG1(H1)-p40	QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGEIPRSGNTYYNEKFKGK VTLTTDKSTRTAYMELRSLTSEDNAVYYCAGKD YGSTYADYWGQGTTLTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGKGGGGSGGGGSIWELKKDVYVVVELD WYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLG SGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYS RFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGA ATLSAERVRGDNKEYEYSVEQCEDSASPAAEESL PIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQ LKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFSVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRA QDRYYSSSWSEWASVPCS (SEQ ID NO: 216)	humanized M43, p40 C177S
2	hM43-Fab(H1)-p40	QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGEIPRSGNTYYNEKFKGK VTLTTDKSTRTAYMELRSLTSEDNAVYYCAGKD YGSTYADYWGQGTTLTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGGSGGGGSIWEL KKDVYVVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKG GEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKT	humanized M43, p40 C177S

		LRCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVEQCEDSASPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGSGLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 217)	
3	hM43-ScFv(vH1-vL4)-p40	QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGISWVRQRTGQGLEWIGEYIPRSGNTYYNEKFKGKVTLTDDKSTRTAYMELRSLTSEDNAVYYCAGKDYGSTYADYWGQGTTLTVSSGGGGSGGGSGGGGSQIVLTQSPATLSASPERVTMTCSASSSVSSRYLHWYQQKSGASPRLWIYGTSNLAGVPARFSGSGPGTSTYTLTISSVEPEDAATYYCQYHSDPLTFGQGTLELKGSGGGSGGGSGIWEKKDVYVVELDWYDAPGEMVVLTCDTPEEDGITWTLTDSSEVLGSGKTLTIQVKEFGDAGQYCHKGGEVLSHSLLLHKKEDGIWSTDILKDKQEPKNTFLRCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVEQCEDSASPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGSGLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 218)	humanized M43, p40 C177S
4	hM43-ScFv(vL4-vH1)-p40	QIVLTQSPATLSASPERVTMTCSASSSVSSRYLHWYQQKSGASPRLWIYGTSNLAGVPARFSGSGPGTSTYTLTISSVEPEDAATYYCQYHSDPLTFGQGTLELKGSGGGSGGGSGGGSGQVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGISWVRQRTGQGLEWIGEYIPRSGNTYYNEKFKGKVTLTDDKSTRTAYMELRSLTSEDNAVYYCAGKDYGSTYADYWGQGTTLTVSSGGGGSGGGSGIWEKKDVYVVELDWYDAPGEMVVLTCDTPEEDGITWTLTDSSEVLGSGKTLTIQVKEFGDAGQYCHKGGEVLSHSLLLHKKEDGIWSTDILKDKQEPKNTFLRCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVEQCEDSASPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGSGLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 219)	humanized M43, p40 C177S
5	hM43-Fab(H1)-Fc (knob)	QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGISWVRQRTGQGLEWIGEYIPRSGNTYYNEKFKGKVTLTDDKSTRTAYMELRSLTSEDNAVYYCAGKDYGSTYADYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGGGGSGGGGSEPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALGAPIEKISKAKGQPREPQVCTLPPSR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGN	humanized M43, Knob T366W, human IgG1, LALA-PG, effector function null

		VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 220)	
6	hM43-ScFv(vH1-vL4)-Fc(knob)	QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGEIYPRSGNTYYNEKFKGK VTLTDDKSTRTAYMELRSLTSEDVAVYYCAGKD YGSTYADYWGQGTTLTVSSGGGSGGGGSGGGG SQIVLTQSPATLSASPGERVMTCSASSSVSSRYL HWYQQKSGASPRWLYGTSLNLSGVPARFSGSGP GTSYTLTISSVEPEDAATYYCQYHSDPLTFGQGT KLELKGSGGGSGGGGSEPKSSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTI SKAKGQPREPQVCTLPSSREEMTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO: 221)	humanized M43, Knob T366W, human IgG1, LALA-PG, effector function null
7	hM43-ScFv(vL4-vH1)-Fc (knob)	QIVLTQSPATLSASPGERVMTCSASSSVSSRYLH WYQQKSGASPRWLYGTSLNLSGVPARFSGSGPG TSYTLTISSVEPEDAATYYCQYHSDPLTFGQGTK LELKGSGGGSGGGGSGGGGSGVQLVQSGAEVAKP GASVNLNCKASGYTFTSYGISWVRQRTGQGLEWI GEIYPRSGNTYYNEKFKGKVTLTDDKSTRTAYME LRSLTSEDVAVYYCAGKDYGSTYADYWGQGTTL TVSSGGGSGGGGSEPKSSDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTI KAKGQPREPQVCTLPSSREEMTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO: 222)	humanized M43, Knob T366W, human IgG1, LALA-PG, effector function null
8	p35-hM43-ScFv(vH1-vL4)2	RNLPVATPDPMFPCLLHHSQNLLRAVDNMLQKA RQTLEFYPTSEEIDHEDITDKTSTVEACLPLELT KNESKLNRETSFITNGSCLASRKTSFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNML AVIDELMQALNFNSETVPQKSLEEDFYKTKIKL CILLHAFRIRAVTINRVMSYLNASGGGSGGGGSG QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGEIYPRSGNTYYNEKFKGK VTLTDDKSTRTAYMELRSLTSEDVAVYYCAGKD YGSTYADYWGQGTTLTVSSGGGSGGGGSGGGG SQIVLTQSPATLSASPGERVMTCSASSSVSSRYL HWYQQKSGASPRWLYGTSLNLSGVPARFSGSGP GTSYTLTISSVEPEDAATYYCQYHSDPLTFGQGT KLELKGSGGGSGGGGSGGGGSGGGGSGGGSGG QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGEIYPRSGNTYYNEKFKGK VTLTDDKSTRTAYMELRSLTSEDVAVYYCAGKD YGSTYADYWGQGTTLTVSSGGGSGGGGSGGGG SQIVLTQSPATLSASPGERVMTCSASSSVSSRYL HWYQQKSGASPRWLYGTSLNLSGVPARFSGSGP GTSYTLTISSVEPEDAATYYCQYHSDPLTFGQGT KLELKGSGSLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 223)	humanized M43, p35 S27D, C74K, D188N

9	p35-hM43- ScFv(vL4-vH1)2	RNLPVATPDPGMFPCLLHHSQNLLRAVDNMLQKA RQTLEFYPTCTSEEIDHEDITKDKTSTVEACLPLELT KNESKLSNRETSFITNGSCLASRKTSFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNML AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKL CILLHAFRIRAVTINRVMSYLNASGGGGSGGGGS QIVLTQSPATLSASPGERVTMTCSASSSVSSRYLH WYQQKSGASPRLWIYGTSNLASGVPARFSGSGPG TSYTLTISSVEPEDAATYYCQQYHSDPLTFGQGTK LELKGSGGGSGGGSGGGSGQVQLVQSGAEVAKP GASVNLNCKASGYTFTSYGISWVRQRTGQGLEWI GEIYPRSGNTYYNEKFKGKVTLTDDKSTRTAYME LRLTSEDNAVYYCAGKDYGSTYADYWGQGTTL TVSSGGSGGGSGGGSGGGSGGGSGGGSGGGG IVLTQSPATLSASPGERVTMTCSASSSVSSRYLHW YQQKSGASPRLWIYGTSNLASGVPARFSGSGPGT SYTLTISSVEPEDAATYYCQQYHSDPLTFGQGTKL ELKGSGGGSGGGSGGGSGQVQLVQSGAEVAKPG ASVNLNCKASGYTFTSYGISWVRQRTGQGLEWIG EIYPRSGNTYYNEKFKGKVTLTDDKSTRTAYMEL RSLTSEDNAVYYCAGKDYGSTYADYWGQGTTLT VSSGGSGGLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 224)	humanized M43, p35 S27D, C74K, D188N
10	hM43-IgG1(H1)- scIL12	QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGIEIYPRSGNTYYNEKFKGK VTLTDDKSTRTAYMELRSLTSEDNAVYYCAGKD YGSTYADYWGQGTTLTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG FYPYSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGKGGGGSGGGSGIWEKKDQVYVVELD WYPDAPGEMVVLTCDTPEEDGITWTLDSSEVLG SGKTLTIQVKEFGDAGQYCHKGGEVLSHSLLLL HKKEDGIWSTDILKDKQEPKNTFLRCEAKNYSG RFTCWVLTITSTDLTFSVKSSRGSSDPQGVTCGA ATLSAERVRGDNKEYEYSVEQCQEDSACPAAEESL PIEVMVDAVHKLKYENYTSSFFIRDIKPDPPKNLQ LKPLKNSRQVEVSWEPDTWSTPHSYFSLTFCVQ VQGKSKREKKDRVFTDKTSATVICRKNASISVRA QDRYYSSSWSEWASVPCSGSGGGSGGGSGGRNLPV ATPDPGMFPCLLHHSQNLLRAVSNMLQKARQTLEF YPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCL NSRETSFITNGSCLASRKTSFMMALCLSIYEDLK MYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDE LMQALNFNSETVPQKSSLEEDFYKTKIKLCILLH AFRIRAVTIDRVMSYLNAS (SEQ ID NO: 225)	humanized M43 LALA, p40-p35
11	hM43-Fab(H1)- scIL12	QVQLVQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGIEIYPRSGNTYYNEKFKGK VTLTDDKSTRTAYMELRSLTSEDNAVYYCAGKD YGSTYADYWGQGTTLTVSSASTKGPSVFPLAPSS	humanized M43, p40- p35

		KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGSGGGGSIWEL KKDVYVVELDWYDPDAPGEMVVLTCDTPEEDGIT WTLDSSEVLGSGKTLTIQVKEFGDAGQYTCHKG GEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTF LRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSS DPQGVTCGAATLSAERVRGDNKEYEYSVEQCED SACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRD IIPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPH SYFSLTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCSGSGGG GSGGRNLPVATPDGPMFPCLLHHSQNLLRAVSNM LQKARQTLFYPCTSEEIDHEDITKDKTSTVEACL PLELTKNESCLNSRETSFITNGSCLASRKTSFMMMA LCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFL DQNMLAVIDELMQALNFNSETVPQKSSLEEPDFY KTKIKLCILLHAFRIRAVTIDRVMSYLNASGGSGG HHHHHHGGSGLNDIFEAQKIEWHE (SEQ ID NO: 226)	
12	hM43-IgG1(H4)- p40	QVQLQQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGEYIPRSGNTYYNEKFKGK ATLTADKSTRTAYMELRSLTSEDVAVYFCAGKDY GSTYADYWGQGTTLTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIK AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKS LSLSPGKGGGSGGGGSIWELKKDVYVVELDWY PDAPGEMVVLTCDTPEEDGITWTLDSSEVLGSG KTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHK KEDGIWSTDILKDQKEPKNKTLRCEAKNYSGRF TCWWLTITISTDLTFSVKSSRGSSDPQGVTCGAAT LSAERVRGDNKEYEYSVEQCEDSASPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIIPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQD RYYSSSWSEWASVPCS (SEQ ID NO: 227)	humanized M43, p40 C177S
13	hM43-Fab(H4)- p40	QVQLQQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGEYIPRSGNTYYNEKFKGK ATLTADKSTRTAYMELRSLTSEDVAVYFCAGKDY GSTYADYWGQGTTLTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHNKPSNTKVDKKVEPKSCGGGSGGGGSIWELK KDVYVVELDWYDPDAPGEMVVLTCDTPEEDGITW TLDSSEVLGSGKTLTIQVKEFGDAGQYTCHKGG EVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTL RCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSD PQGVTCGAATLSAERVRGDNKEYEYSVEQCEDS ASPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDI	humanized M43, p40 C177S

		KPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHS YFSLTFSVQVQGKSKREKKDRVFTDKTSATVICR KNASISVRAQDRYYSSSWSEWASVPCSGGSGLND IFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 228)	
14	hM43-ScFv(vH4- vL4)-p40	QVQLQQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGEYPRSGNTYYNEKFKGK ATLTADKSTRTAYMELRSLTSEDNAVYFCAGKDY GSTYADYWGQGTTLTVSSGGGSGGGGSGGGGS QIVLTQSPATLSASPGERVMTCSASSSVSSRYLH WYQQKSGASPRLWIYGTSNLASGVPARFSGSGPG TSYTLTISSVEPEDAATYYCQQYHSDPLTFGQGTK LELKGGGSGGGGSIWELKKDVYVVELDWYPDA PGEMVVLTCDTPEEDGITWTLTDSSEVLGSGKTL TIQVKEFGDAGQYTCHKGGEVLSHSLLLHKKED GIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCW WLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAE RVRGDNKEYEYSVEQEDSASPAAEESLPIEVMV DAVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLK NSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKS KREKKDRVFTDKTSATVICRKNASISVRAQDRYY SSSWSEWASVPCSGGSGLNDIFEAQKIEWHEGG SHHHHHH (SEQ ID NO: 229)	humanized M43, p40 C177S
15	hM43-ScFv(vL4- vH4)-p40	QIVLTQSPATLSASPGERVMTCSASSSVSSRYLH WYQQKSGASPRLWIYGTSNLASGVPARFSGSGPG TSYTLTISSVEPEDAATYYCQQYHSDPLTFGQGTK LELKGGGSGGGGSGGGGSGVQLQQSGAEVAKP GASVNLNCKASGYTFTSYGISWVRQRTGQGLEWI GEIYPRSGNTYYNEKFKGKATLTADKSTRTAYME LRSLTSEDNAVYFCAGKDYGSTYADYWGQGTTL TVSSGGGSGGGGSIWELKKDVYVVELDWYPDA PGEMVVLTCDTPEEDGITWTLTDSSEVLGSGKTL TIQVKEFGDAGQYTCHKGGEVLSHSLLLHKKED GIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCW WLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAE RVRGDNKEYEYSVEQEDSASPAAEESLPIEVMV DAVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLK NSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQGKS KREKKDRVFTDKTSATVICRKNASISVRAQDRYY SSSWSEWASVPCSGGSGLNDIFEAQKIEWHEGG SHHHHHH (SEQ ID NO: 230)	humanized M43, p40 C177S
16	hM43-Fab(H4)- Fc (knob)	QVQLQQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGEYPRSGNTYYNEKFKGK ATLTADKSTRTAYMELRSLTSEDNAVYFCAGKDY GSTYADYWGQGTTLTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCGGGSGGGGSEPKSS DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALGAPIEKTIKAKGQPREPQVCTLPPSRE EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 231)	humanized M43, Knob T366W, human IgG1, LALA-PG, effector function null

17	hM43-ScFv(vH4-vL4)-Fc(knob)	QVQLQQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGIEIYPRSGNTYYNEKFKGK ATLTADKSTRTAYMELRSLTSEDNAVYFCAGKDY GSTYADYWGQGTTLTVSSGGGGSGGGSGGGGS QIVLTQSPATLSASPERVTMTCSASSSVSSRYLH WYQQKSGASPRLWIYGTSNLASGVPARFSGSGPG TSYTLTISSVEPEDAATYYCQQYHSDPLTFGQGTK LELKGGGGSGGGGSEPKSSDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTIS KAKGQPREPQVCTLPSSREEMTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFF LYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO: 232)	humanized M43, Knob T366W, human IgG1, LALA-PG, effector function null
18	hM43-ScFv(vL4-vH4)-Fc (knob)	QIVLTQSPATLSASPERVTMTCSASSSVSSRYLH WYQQKSGASPRLWIYGTSNLASGVPARFSGSGPG TSYTLTISSVEPEDAATYYCQQYHSDPLTFGQGTK LELKGGGGSGGGGSGGGGSGVQLQQSGAEVAKP GASVNLNCKASGYTFTSYGISWVRQRTGQGLEWI GEIYPRSGNTYYNEKFKGKATLTADKSTRTAYME LRSLTSEDNAVYFCAGKDYGSTYADYWGQGTTL TVSSGGGGSGGGGSEPKSSDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTIS KAKGQPREPQVCTLPSSREEMTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFF LYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO: 233)	humanized M43, Knob T366W, human IgG1, LALA-PG, effector function null
19	p35-hM43-ScFv(vH4-vL4)2	RNLPVATPDPGMFPCLLHHSQNLLRAVDNMLQKA RQTLEFYPTSEEIDHEDITKDKTSTVEACLPLELT KNESKLNSRETSFITNGSCLASRKTSFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNML AVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKL CILLHAFRIRAVTINRVMSYLNASGGGGSGGGGS QVQLQQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGIEIYPRSGNTYYNEKFKGK ATLTADKSTRTAYMELRSLTSEDNAVYFCAGKDY GSTYADYWGQGTTLTVSSGGGGSGGGSGGGGS QIVLTQSPATLSASPERVTMTCSASSSVSSRYLH WYQQKSGASPRLWIYGTSNLASGVPARFSGSGPG TSYTLTISSVEPEDAATYYCQQYHSDPLTFGQGTK LELKGGSGGGGSGGGSGGGGSGGGGSGGGSGGQ VQLQQSGAEVAKPGASVNLNCKASGYTFTSYGIS WVRQRTGQGLEWIGIEIYPRSGNTYYNEKFKGKA TLTADKSTRTAYMELRSLTSEDNAVYFCAGKDY STYADYWGQGTTLTVSSGGGGSGGGSGGGGSQ IVLTQSPATLSASPERVTMTCSASSSVSSRYLHW YQQKSGASPRLWIYGTSNLASGVPARFSGSGPGT SYTLTISSVEPEDAATYYCQQYHSDPLTFGQGTKL ELKGGGSLNDIFEAKIEWHEGGSHHHHHH (SEQ ID NO: 234)	humanized M43, p35 S27D, C74K, D188N
20	p35-hM43-ScFv(vL4-vH4)2	RNLPVATPDPGMFPCLLHHSQNLLRAVDNMLQKA RQTLEFYPTSEEIDHEDITKDKTSTVEACLPLELT KNESKLNSRETSFITNGSCLASRKTSFMMALCLSSI	humanized M43, p35 S27D, C74K, D188N

		YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNML AVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKL CILLHAFRIRAVTINRVMSYLNASGGGSGGGGS QIVLTQSPATLSASPGERVMTCSASSSVSSRYLH WYQQKSGASPRLWIYGTSNLASGVPARFSGSGPG TSYTLTISSVEPEDAATYYCQQYHSDPLTFGQGTK LELKGGGSGGGSGGGGSQVQLQQSGAEVAKP GASVNLNCKASGYTFTSYGISWVRQRTGQGLEWI GEIYPRSGNTYYNEKFKGKATLTADKSTRTAYME LRSLTSEDNAVYFCAGKDYGSTYADYWGGQTTL TVSSGGSGGGSGGGSGGGSGGGSGGGSGGGQ IVLTQSPATLSASPGERVMTCSASSSVSSRYLHW YQQKSGASPRLWIYGTSNLASGVPARFSGSGPGT SYTLTISSVEPEDAATYYCQQYHSDPLTFGQGTKL ELKGGGSGGGSGGGSGGGGSQVQLQQSGAEVAKPG ASVNLNCKASGYTFTSYGISWVRQRTGQGLEWIG EIYPRSGNTYYNEKFKGKATLTADKSTRTAYMEL RSLTSEDNAVYFCAGKDYGSTYADYWGGQTTLT VSSGGSLNDIFEAQKIEWHEGGSHHHHHH (SEQ ID NO: 235)	
21	hM43-IgG1(H4)- scIL12	QVQLQQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGEIYPRSGNTYYNEKFKGK ATLTADKSTRTAYMELRSLTSEDNAVYFCAGKDY GSTYADYWGGQTTLTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NPKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIK AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGKGGGSGGGSGGGSIWELKDVYVVELDWY PDAPGEMVVLTCDTPEEDGITWTLDSSEVLGSG KTLTIQVKEFGDAGQYTCCHKGGEVLSHSLLLLHK KEDGIWSTDILKDKQEPKNTFLRCEAKNYSGRF TCWWLTISTDLTFSVKSSRGSSDPQGVTCGAAT LSAERVRGDNKEYEYSVEQCEDSACPAAEESLPIE VMVDAVHKLKYENYTSSFFIRDIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQD RYYSSSWSEWASVPCSGSGGGSGGGNLPVATP DPGMFPLHHSQNLLRAVSNMLQKARQTLEFYP CTSEEDHEDITKDKTSTVEACLPLELTKNESCLNS RETSFITNGSCLASRKTSFMMALCLSSIEDLKMY QVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELM QALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFR IRAVTIDRVMSYLNAS (SEQ ID NO: 236)	humanized M43 LALA, p40-p35
22	hM43-Fab(H4)- scIL12	QVQLQQSGAEVAKPGASVNLNCKASGYTFTSYGI SWVRQRTGQGLEWIGEIYPRSGNTYYNEKFKGK ATLTADKSTRTAYMELRSLTSEDNAVYFCAGKDY GSTYADYWGGQTTLTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NPKPSNTKVDKKVEPKSCGGGSGGGSGGGSIWELK	humanized M43, p40- p35

		KDYYVELDWYPDAPGEMVVLTCDTPEEDGITW TLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGG EVL SHSLLLLHKKEDGIWSTDILKDQKEPKNKTF RCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSD PQGVTCGAATLSAERVGRDNKEYEYSVEQEDS ACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDII KPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHS YFSLTFCVQVQGKSKREKKDRVFTDKTSATVICR KNASISVRAQDRYYSSSWSEWASVPCSGGSGGGG SGGRNLPVATPDPMFPCLLHHSQNLRLRAVSNNL QKARQTLEFYPCSTSEIDHEDITKDKTSTVEACLP LELTKNESCLNSRETSFITNGSCLASRKTSFMMAL CLSSIEDLKMYQVEFKTMNAKLLMDPKRQIFLD QNMLAVIDELMQALNFNSETVPQKSLEEDFYK TKIKLCILLHAFRIRAVTIDRVMSYLNASGGSGGH HHHHHGGSLNDIFEAQKIEWHE (SEQ ID NO: 237)	
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Example 6 – Additional uPAR Targeting Fusion Proteins

Design of Fusion Proteins Example 6

5 Fusion protein constructs designed and/or synthesized in accordance with Example 6 are found in **Table 31**. Some of these amino acid sequences found in **Table 31** are used as controls for the following experimental examples. Sequences involving mouse proteins or human proteins modified for better binding in mouse experiments are included for the purpose of testing in mouse models and mouse cell systems. Any of the constructs using mouse

10 proteins or human proteins modified for mouse experiments may be substituted with fully human proteins.

For expression in mammalian cells, an N-terminal leader sequence was added to certain fusion protein constructs found in **Table 31**. Also, 6xHis and FLAG tags and avidin polypeptides were added to certain fusion protein constructs found in **Table 31** for ease of

15 purification during recombinant procedures.

It is understood that the constructs in **Table 31** of fusion proteins of Example 6 may be modified to, for example, modify amino acid compositions or length of the linker molecules between polypeptide molecules without loss of the desired bioactivity. The order of fusion of the various polypeptides may be changes such that, for example, that the uPAR targeting domain region may be at the N-terminus or C-terminus of the cytokine or immune

20 checkpoint modulator portion of the fusion proteins or that other fusion proteins may be added to the constructs in **Table 31** via optional linkers such as an Fc fusion protein or a

human serum albumin (HAS) fusion protein. Abbreviations in **Table 31** include: single chain (sc); circularly permuted (cp) scrambled sequence (ss).

Table 31 – Amino Acid Sequences Of Polypeptides Described In Example 6

Description	Amino Acid Sequence/SEQ ID NO
Human uPA UniProt: P00749	MRALLARLLLCVLVVS DSKGSNELHQVPSNCDCLNGGTCVS NKYFSNIHWCNCPKKFGGQHCEIDKSKTCYEGNGHFYRGKA STD TMGRPCLPWN SATVLQQTYHAHRSDALQLGLGKHNYCR NPDNRRRPWCYVQVGLKPLVQECMVHDCADGKKPSSPPEEL KFQCGQKTLRPRFKIIGGEFTTIENQPWF AAIYRRHRGGSVTY VCGGSLISPCWVISATHCFIDYPKKEDYIVYLGRSRLNSNTQG EMKFEVENLILHKDYSADTLAHHNDIALLKIRSKEGRCAQPSR TIQTICLPSMYNDPQFGTSCEITGFGKENSTDYLYPEQLKMTV VKLISHRECQQPHYYGSEVTTKMLCAADPQWKTDSCQGD SG GPLVCSLQGRMTLTGIVSWGRGCALDKDPGVYTRVSHFLPWI RSHTKEENGLAL (SEQ ID NO: 90)
Human ATF	SNELHQVPSNCDCLNGGTCVSYKYFSNIHRCNCPKKFGGQHC EIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWN SATVLQQT YHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPLV QECMVHDCA (SEQ ID NO: 91)
Mouse uPA UniProt: P06869	MKVWLASLFLCALVVKNSEGGSVLGAPDESNCGCQNGGVC VSYKYFSRIRRCSCPRKFQGEHCEIDASKTCYHNGGDSYRGK ANTDTKGRPCLAWNAPAVLQKPYN AHRPD AISLGLGKHNYC RNPDNQKRPWCYVQIGLRQFVQECMVHDCSLSKKPSSSV DQ QGFQCGQKALRPRFKIVGGEFTEVENQPWF AAIYQKNKGGS P PSFKCGGSLISPCWVASA AHCFIQLPKKENYV VYLGQSKES SY NPGEMKFEVEQLILHEYYREDSLAYHNDIALLKIRTSTGQCAQ PSRSIQTICLPPRFTDAPFGSDCEITGFGKESESDYL YPKNLKMS VVKLV SHEQCMQPHYYGSEINYKMLCAADPEWKTDSCKGDS GGPLICNIEGRPTLSGIVSWGRGCAEKNKPGVYTRVSHFLDWI QSHIGEEKGLAF (SEQ ID NO: 93)
human cpIL-2:IL-2Ra fusion	SKNFHLRPRDLISNINIVLELKGSETTFMCEYADETATIVEFL NRWITFSQSIISTLTGGSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTRMLTFKFYMPKKATEL KHLQCLEELKPLEEVLNL AQSGGGGSELCD DDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTP QP EEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATER IYHFVVGQMVYYQC VQGYRALHRGPAESVCKMTHGKTRWT QPQLICTG (SEQ ID NO: 94)
human IL-15Ra:cpIL- 15	ITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKRKAGTSSLT ECVLNKATNVAHWTTPSLKCIRDGGSELEEKNIKEFLQSFVHI VQMFINGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHP SCKVTAMKCFLELQVISLESGDASIHDVTENLILANNSLSSN GNVTESGCKEC* (SEQ ID NO: 95)

cp murine IL-2-IL-2Ra fusion	SSKSFQLEDAENFISNIRVTVVKLKGSNDNTFECQFDDDESATVVDFLRRWIAFCQSIISTSPQGGSSSTQQQQQHLEQLLMDLQELLSRMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSGGGGSELCLYDPPEVPNATFKALS YKNGTILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQT TTDMMQKPTQSMHQENLTGHCREPPPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCKGTGWTQPQLTCVD*(SEQ ID NO: 96)
Mouse IL-15:15Ra fusion	TTCPPPVSIHADIRVKNYSVNSRERYVCNSGFKRKAGTSTLIECVINKNTNVAHWTTPSLKCIRDggsELEEKTFTEFLQSFIRIVQMFINGggsNWIDVRYDLEKIESLIQSIHIDTTLYTDSDFHPSCKV TAMNCFLELQVILHEYSNMTLNETVRNVLYLANSTLSSNKNVAESGCKEC**(SEQ ID NO: 97)
Human uPA-ATF:human cpIL-2:IL-2Ra	SNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPKKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQTYHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPLVQECMVHDCAGGSGGSKNFHLRPRDLISNINVIVLELKGETTFMCEYADETATIVEFLNRWITFSQSIISTLTGGSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQGGSGGSELCDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLP GHCREPPPWENEATERIYHFVVGQMVYYQCVCQGYRALHRGPAESVCKMTHGKTRWTQPQLICTG (SEQ ID NO: 98)
Human cpIL-2:IL-2Ra:Human uPA-ATF	SKNFHLRPRDLISNINVIVLELKGETTFMCEYADETATIVEFLNRWITFSQSIISTLTGGSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQGGSGGSELCDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLP GHCREPPPWENEATERIYHFVVGQMVYYQCVCQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGGGSGGSNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPKKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQTYHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPLVQECMVHDCA (SEQ ID NO: 99)
Human uPA-ATF:human IL-15Ra:cpIL-15	SNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPKKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQTYHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPLVQECMVHDCAGGSGGITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGGSELEEKNIKEFLQSFVHIVQMFINGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLES GDASIHD TVENLILANNSLSSNGNVTESGCKEC (SEQ ID NO: 100)
mouse cpIL-2:IL-2Ra:mouse uPA-ATF	SKSFQLEDAENFISNIRVTVVKLKGSNDNTFECQFDDDESATVVDFLRRWIAFCQSIISTSPQGGSSSTQQQQQHLEQLLMDLQELLSRMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRH

	VLDLTQ <u>GSGGG</u> SELCLYDPPEVPNATFKALSYKNGTILNCEC KRGFRRLKELVYMRCLGNSWSSNCQCTSN SHDKSRKQVTAQ LEHQKEQQT TTD MQKPTQSMHQENLTGHCREPPPWKHEDSK RIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQ PQLTCVDggsggGSVLGAPDESNCGCQNGGVCVSYKYFSRIRR CSCPRKFQGEHCEIDASKTCYHGNNGDSYRGKANTDTKGRPCL AWNAPAVLQKPYNAPHRPDAISLGLGKHNYCRNPDNQRKPW CYVQIGLRQFVQECCMVHDC (SEQ ID NO: 101)
uPA-ATF:mouse IL-15Ra:cpIL-15	GSVLGAPDESNCGCQNGGVCVSYKYFSRIRRCSCPRKFQGEH CEIDASKTCYHGNNGDSYRGKANTDTKGRPCLAWNAPAVLQK PYNAPHRPDAISLGLGKHNYCRNPDNQRKPWCYVQIGLRQFV QECCMVHDCGGSGGTTCPPPVSIHADIRVKNYSVNSRERYVC NSGFKRKAGTSTLIECVINKNTNVAHWTPSLKCIRDGGSELE EKTTFEFLQSFIRIVQMFINGGGSNWIDVRDLEKIESLIQSIHI DTTLYTDSDFHPSCKVTAMNCFLELQVILHEYSNMTLNETV RNVLYLANSTLSSNKNVAESGCKEC (SEQ ID NO: 102)
Human uPA-ATF (control molecule)	SNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPKKFGGQH CEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWN SATVLQQ TYHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPL VQECCMVHDC (SEQ ID NO: 103)
Mouse uPA-ATF (control molecule)	GSVLGAPDESNCGCQNGGVCVSYKYFSRIRRCSCPRKFQGEH CEIDASKTCYHGNNGDSYRGKANTDTKGRPCLAWNAPAVLQK PYNAPHRPDAISLGLGKHNYCRNPDNQRKPWCYVQIGLRQFV QECCMVHDC (SEQ ID NO: 104)
Mouse uPA-ATF:mouse cpIL-2:IL-2Ra	GSVLGAPDESNCGCQNGGVCVSYKYFSRIRRCSCPRKFQGEH CEIDASKTCYHGNNGDSYRGKANTDTKGRPCLAWNAPAVLQK PYNAPHRPDAISLGLGKHNYCRNPDNQRKPWCYVQIGLRQFV QECCMVHDCGGSGGSKSFQLEDAENFISNIRVTVVKLKGSNT FECQFDDSATVVDLRRWIAFCQSIISTSPQGGSSSTQQQQH LEQLLMDLQELL SRMENYRN LKLPRMLTFKFYLPKQATELKD LQCLEDELGPLRHVLDLTQSGGGSELCLYDPPEVPNATFKA LSYKNGTILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSN SHDKSRKQVTAQLEHQKEQQT TTD MQKPTQSMHQENLTGH CREPPPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISIC KMKCGKTGWTQPQLTCVD (SEQ ID NO: 105)
human cpIL-2:IL-2Ra:human IgG1 Fc:Human uPA-ATF* Molecule may be modified to reduce ADCC by mutating underlined LL to AA.	SKNFHLRPRDLISNINIVLELKGSETTFMCEYADETATIVEFL NRWITFSQSIISTLTGGSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTRMLTFKFYMPKKATELKHLCLEELKPLEEVLNL AQSGGGSELCDDDPPEIPHATFKAMAYKEGTM LNCECKRG FRRIKSGSLYMLCTGNSSSHSSWDNQCQCTSSATRNTTKQVTP QPPEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATER IYHFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWT QPQLICTGgggsEPKSSDKTHTCPPCPAPE <u>LL</u> GGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY

	PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFNCSVMHEALHNHYTQKSLSLSPGKGGSGGGSNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPKKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQTYYHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPLVQECMVHDC (SEQ ID NO: 106)
Human uPA-ATF: human IgG1 Fc :h cp IL-15 Molecule may be modified to reduce ADCC by mutating underlined LL to AA.	SNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPKKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQTYYHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPLVQECMVHDCAGGSGGEPKSSDKTHTCPPCPAPE <u>LL</u> GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFNCSVMHEALHNHYTQKSLSLSPGK _{gs} ITCPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTPSLKCI RDGGSELEEKNIKEFLQSFVHIVQMFI NGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLESGDASIHTVENLILANNSLSSNGNVTESGCKEC (SEQ ID NO: 107)
mouse cpIL-2:IL-2Ra: mouse IgG1:mouse uPA-ATF	SKSFQLEDAENFISNIRVTVVKLKGS DNTFECQFDDESATVVD FLRRWIAFCQSIISTSPQGGSSSTQQQQHLEQLLMDLQELLSRMENYRNKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSGSGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKELVYMRC LGNSWSSNCQCTSN SHDKSRKQVTAQLEHQKEQQT TDMQKPTQSMHQENLTGHCREPPPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVD _{gs} VPRD _{gs} GCKPCICTVPEVSSVFIFPPKPKDVLITLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQIMNTNGSYFVYSKLVQKSNWEAGNTFTCSVLHEGLHNHHTTEKSLSHSPGK _{gsggs} GSVLGAPDESNCGCQNGGVCVSYKYFSRIRRCSCPRKFQGEHCEIDASKTCYHGNGDSYRGKANTDTKGRPCLAWNAPAVLQKPYN AHRPDAISLGLGKHNYCRNPDNQKRPWCYVQIGLRQFVQECMVHDC (SEQ ID NO: 108)
Mouse uPA-ATF:Fc:m cp IL-15	GSVLGAPDESNCGCQNGGVCVSYKYFSRIRRCSCPRKFQGEHCEIDASKTCYHGNGDSYRGKANTDTKGRPCLAWNAPAVLQKPYN AHRPDAISLGLGKHNYCRNPDNQKRPWCYVQIGLRQFVQECMVHDC _{gsggs} VPRD _{gs} GCKPCICTVPEVSSVFIFPPKPKDVLITLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQIMNTNGSYFVYSKLVQKSNWEAGNTFTCSVLHEGLHNHHTTEKSLSHSPGK _{ggs} TTCPPPVSIEHADIR

	VKNYSVNSRERYVCNSGFKRKAGTSTLIECVINKNTNVAHWT TPSLKCIRD ^{ggs} ELEEKTFTEFLQSFIRIVQMFIN ^{ggs} NWIDVRY DLEKIESLIQSIHIDTTLYTDSDFHPSCCKVTAMNCFLEELQVILH EYSNMTLNETVRNVLYLANSTLSSNKNVAESGCKEC (SEQ ID NO: 109)
human cpIL-2:IL-2Ra fusion :Human uPA- ATF (N22Y; W30R)	SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFL NRWITFSQSIISTLTGGSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL AQQSGGGSELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTP QPPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATER IYHFVVGQMVYYQCQVQGYRALHRGPAESVCKMTHGKTRWT QPQLICTGGGSGGSNELHQVPSNCDCLNGGTCVSYKYFSNIH RCNCPKKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRP CLPWNSATVLQQTYHAHRSDALQLGLGKHNYCRNPDNRRRP WCYVQVGLKPLVQECMVHDCA** (SEQ ID NO: 110)
human cpIL-2:IL- 2Ra- P25-	SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFL NRWITFSQSIISTLTGGSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL AQQSGGGSELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTP QPPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATER IYHFVVGQMVYYQCQVQGYRALHRGPAESVCKMTHGKTRWT QPQLICTGGGSGGGGSGGAESTYHHL ^{SLGYMYTLN} GGSHHH HHHGSGSLNDIFEAQKIEWHE (SEQ ID NO: 111)
human cpIL-2:IL- 2Ra- ssP25-	SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFL NRWITFSQSIISTLTGGSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL AQQSGGGSELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTP QPPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATER IYHFVVGQMVYYQCQVQGYRALHRGPAESVCKMTHGKTRWT QPQLICTGGGSGGGGSGGNYHYLESSMTALYTLGH (SEQ ID NO: 112)
human cpIL-2:IL- 2Ra- M25	SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFL NRWITFSQSIISTLTGGSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL AQQSGGGSELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTP QPPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATER IYHFVVGQMVYYQCQVQGYRALHRGPAESVCKMTHGKTRWT QPQLICTGGGSGGGGSGGPRYQHIGLVAMFRQNTG (SEQ ID NO: 113)
human cpIL-2:IL- 2Ra- ssM25	SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFL NRWITFSQSIISTLTGGSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL

	AQGSGGGSELCDDDPPEIPHATFKAMAYKEGTMLNCECKRG FRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTP QPREEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATER IYHFVVGQMVYYQCQVQGYRALHRGPAESVCKMTHGKTRWT QPQLICTGGGSGGGGSGGHQIPGAYRGVNQRFTML (SEQ ID NO: 114)
<i>anti-CTLA-4 9d9 Mouse IgG kappa light chain</i>	DIRRADIVMTQTTLSPVSLGDQASISCRSSQSIVHSNGNTYLE WYLQKPGQSPKLLIYKVSNRFSGVDPDRFSGSGSGTDFTLKISR VEAEDLGYYCFQGSHPVPTFGGGTKLEIKRADAAPTVSIFPP SSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNS WTDQDSKDSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRENEC (SEQ ID NO: 115)
<i>9d9 Mouse IgG2a heavy chain</i>	EAKLQESGPVLVKPGASVKMSCKASGYTFTDYYMNWVKQS HGKSLEWIGVINPYNGDTSYNQKFKGKATLTVDKSSSTAYME LNSLTSEDSAVYYCARYYGSWFAYWGQGLITVSTAKTTAPS VYPLAPVCGDITGSSVTLGCLVKGYFPEPVTLTWNSGSLSSG VHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTK VDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMI LSPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDY NSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLPAPIERTISK KGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIYVE WTNNGKTELNYKNTEPVLDSGYSYFMYSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFRTPGK** (SEQ ID NO: 116)
<i>anti-CTLA-4 9d9 Mouse IgG2a heavy chain:mouse uPA- ATF</i>	EAKLQESGPVLVKPGASVKMSCKASGYTFTDYYMNWVKQS HGKSLEWIGVINPYNGDTSYNQKFKGKATLTVDKSSSTAYME LNSLTSEDSAVYYCARYYGSWFAYWGQGLITVSTAKTTAPS VYPLAPVCGDITGSSVTLGCLVKGYFPEPVTLTWNSGSLSSG VHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTK VDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMI LSPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDY NSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLPAPIERTISK KGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIYVE WTNNGKTELNYKNTEPVLDSGYSYFMYSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFRTPGKggsggsGSVLGAPDESN CGCQNGGVCVSYKYFSRIRRCSCPRKFQGEHCEIDASKTCYH GNGDSYRGKANTDTKGRPCLAWNAPAVLQKPYNHRPDAIS LGLGKHNYCRNPDNQKRPWCYVQIGLRQFVQECMVHDC (SEQ ID NO: 117)**
<i>US Pat. No: 7858746 B2 4 light J43; anti-PD-1 J43 light chain</i>	YELTQPPSASVNVGETVKITCSGDQLPKYFADWFHQSDQTL QVIYDDNKRPSGIPERISGSSSGTTATLTIRDVRAEDEGDYYCF SGYVDSDSLKYVFGSGTQLTVLGGPKSSPKVTVFPPSPEELRT NKATLVCLVNDFYPGSATVTWKANGATINDGVKTTKPSKQG QNYMTSSYLSLTADQWKSHNRVSCQVTHEGETVEKSLSPA CL** (SEQ ID NO: 118)
<i>US Pat No. 7858746 B2 11</i>	RLLESGGLVKPEGSLKLSCVASGFTFSDYFMSWVRQAPGKG LEWVAHIYTKSYNYATYYSGSVKGRFTISRDDSRSMVYLQM

<i>heavy v J43 using GenBank; AAA82733.1 for CH1,2,3; anti-PD-1 J43 heavy chain</i>	NNLRTE DTATYYCTRDGSGYPSLDFWGQGTQVTVSSATTTaps vyplapacdttsttdvtlglclvkgyfpepvtvswngaltsghvthfsvlhsglyslsssvtvp stwpkqpitchvahasstkvdkkieprtdtdtcnppdpceptcdllggpsvfifppkpk dvlmisltpkitcvvvdvseeepdvqfnwyvnnvedktaqtrqrqynstyrvsvlpikhq dwmsgkvfckvnnnalpspietiskprgqvrpqiytfpppieqtvkdvsvtclvtgflp qdihvewesngqpqpeqnykntqpvl dsdgsyflysklnvpksrwdqgdsftcsvihealh nhhmtktisrslgn** (SEQ ID NO: 119)
<i>anti-PD-1 J43 heavy chain:mouse ATF</i>	RLLESGGGLVKPEGSLKLSCVASGFTFSDYFMSWVRQAPGKG LEWVAHIYTKSYNYATYYSGSVKGRFTISRDDSRSMVYLQM NNLRTE DTATYYCTRDGSGYPSLDFWGQGTQVTVSSATTTaps vyplapacdttsttdvtlglclvkgyfpepvtvswngaltsghvthfsvlhsglyslsssvtvp stwpkqpitchvahasstkvdkkieprtdtdtcnppdpceptcdllggpsvfifppkpk dvlmisltpkitcvvvdvseeepdvqfnwyvnnvedktaqtrqrqynstyrvsvlpikhq dwmsgkvfckvnnnalpspietiskprgqvrpqiytfpppieqtvkdvsvtclvtgflp qdihvewesngqpqpeqnykntqpvl dsdgsyflysklnvpksrwdqgdsftcsvihealh nhhmtktisrslnggsggsGSVLGAPDESNCGCQNGGVCVSYKYFSRI RRCSCPRKFQGEHCEIDASKTCYHGNGDSYRGKANTDTKGRP CLAWNAPAVLQKPYNAHRPDAISLGLGKHNYCRNPDNQKRP WCYVQIGLRQFVQECMVHDC (SEQ ID NO: 120)
mouse ATF: anti-PD-1 J43 heavy chain	GSVLGAPDESNCGCQNGGVCVSYKYFSRI RRCSCPRKFQGEH CEIDASKTCYHGNGDSYRGKANTDTKGRPCLAWNAPAVLQK PYNAHRPDAISLGLGKHNYCRNPDNQKRPWCYVQIGLRQFV QECMVHDCggsggRLLESGGGLVKPEGSLKLSCVASGFTFSDY FMSWVRQAPGKGLEWVAHIYTKSYNYATYYSGSVKGRFTIS RDDSRSMVYLQMNNLRTE DTATYYCTRDGSGYPSLDFWGQ GTQVTVSSATTTapsvyplapacdttsttdvtlglclvkgyfpepvtvswngaltsg vthfsvlhsglyslsssvtvpstwpkqpitchvahasstkvdkkieprtdtdtcnppdpce ptcptdllggpsvfifppkpkdvlmisltpkitcvvvdvseeepdvqfnwyvnnvedktaq etrqrqynstyrvsvlpikhqdwmsgkvfckvnnnalpspietiskprgqvrpqiytfp ppieqtvkdvsvtclvtgflpqdihvewesngqpqpeqnykntqpvl dsdgsyflysklnv pksrwdqgdsftcsvihealnhhmtktisrslgn (SEQ ID NO: 121)
Parent human PD-1 (UniProt: Q15116) extracellular domain	MQIPQAPWPVWVAVLQLGWRPGWFLDSPDRPWNPTTFSPAL LVVTEGDNFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPED RSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAIS LAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV VGVVGGLLGLVLLVWVLAVICSRAARGTIGARRTGQPLKED PSAVPVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPSG MGTSSPARRGSADGPRSAQPLRPEDGHCSWPL (SEQ ID NO: 122)
human uPA-ATF:5AA linker:human PD-1	SNELHQVPSNCDCLNGGTCVSYKYFSNIHRCNCPPKKFGGQHC EIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQT YHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPLV QECMVHDCAGGSGGPGWFLDSPDRPWNPTTFSPALLVVTEGD NATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPEDRSQPGQDC RFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPSPRPAGQFQTLV (SEQ ID NO: 123)

huPA-ATF (N22Y, W30R) : 10AA linker : hPD-1 :	SNELHQVPSNCDCLNGGTCVSYKYFSNIHRCNCPPKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWN SATVLQQTYHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPLVQECMVHDCAGGSGGGSGGPGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLN WYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYL CGAISLAPKAQIKESLRAELRV TERRAEVPTAHPSPSPRPAGQFQTLV (SEQ ID NO: 124)
hPD-1 : 5AA linker : huPA-ATF (N22Y, W30R)	PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLN WYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYL CGAISLAPKAQIKESLRAELRV TERRAEVPTAHPSPSPRPAGQFQTLVGGSGGSGNELHQVPSNCDCLNGGTCVSYKYFSNIHRCNCPPKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWN SATVLQQTYHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPLVQECMVHDC (SEQ ID NO: 125)
hPD-1 : 10AA linker : huPA-ATF (N22Y, W30R) :	PSPRPAGQFQTLVGGSGGGSGGSGNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPPKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWN SATVLQQTYHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPLVQECMVHDC (SEQ ID NO: 126)
<i>mouse ATF: mouse IL-2</i>	GSVLGAPDESNCGCQNGGVCVSYKYFSRIRRCSCPRKFQGEHCEIDASKTCYHGNGDSYRGKANTDTKGRPCLAWNAPAVLQKPYN AHRPDAISLGLGKHNYCRNPDNQKRPWCYVQIGLRQFVQECMVHDCggsggAPTSSSTSSSTA EAQQQQQQQQQQQHLEQLLMDLQELL SRMENYRNKLPRMLTFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTTVVKLKGSDNTFECQFDDESATVVDLRRWIAFCQSIISTSPQ (SEQ ID NO: 127)
<i>mouse ATF: mouse IL-15</i>	GSVLGAPDESNCGCQNGGVCVSYKYFSRIRRCSCPRKFQGEHCEIDASKTCYHGNGDSYRGKANTDTKGRPCLAWNAPAVLQKPYN AHRPDAISLGLGKHNYCRNPDNQKRPWCYVQIGLRQFVQECMVHDCggsggNWIDVRYDLEKIESLIQSIHIDTTLYTDSDFHPSCKVTAMNCFLELQVILHEYSNMTLNETVRNVLYLANSTLSSNKNVAESGCKECEEELEKTFTEFLQSFIRIVQMFINTS (SEQ ID NO: 128)
Linked 4-1BBL Trimer with GSG linkers between monomers	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVA KAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVGLFRVTPEIPAGLPSPRSEGSGREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVA KAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVGLFRVTPEIPAGLPSPRSEGSGREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVA KAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLS

	AGQRLGVHLHTEARARHAWQLTQGATVGLFRVTPEIPAGLP SPRSE (SEQ ID NO: 129)
Linked 4-1BBL trimer with GSG linker between monomers	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSD PGLAGVSLTGGLSYKEDTKELVVAAGVYYYVFFQLELRRVV AGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARN AFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVGL LFRVTPEIPAGLPSPRSESGREGPELSPDDPAGLLDLRQGMFA QLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV AKAGVYYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGA AALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHT EARARHAWQLTQGATVGLFRVTPEIPAGLPSPRSESGREGP ELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLA GVSLTGGLSYKEDTKELVVAAGVYYYVFFQLELRRVVAGEG SGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQ GRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVGLFRVT PEIPAGLPSPRSE (SEQ ID NO: 130)
uPA-ATF – 5 Linker – Linked 4- 1BBL Trimer	SNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPKKFGGQHC EIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQTY HAHRSDALQLGLGKHNCRNPDNRRRPWCYVQVGLKPLVQE CMVHDCAGGSGGREGPELSPDDPAGLLDLRQGMFAQLVAQN VLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAAGVY YVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALT DLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHA WQLTQGATVGLFRVTPEIPAGLPSPRSESGREGPELSPDDPA GLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGL SYKEDTKELVVAAGVYYYVFFQLELRRVVAGEGSGSVSLALH LQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAG QRLGVHLHTEARARHAWQLTQGATVGLFRVTPEIPAGLPSPR SESGREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLS WYSDPGLAGVSLTGGLSYKEDTKELVVAAGVYYYVFFQLELR RVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEAR NSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATV LGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 131)
Linked 4-1BBL Trimer – 5 Linker – uPA-ATF	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDP GLAGVSLTGGLSYKEDTKELVVAAGVYYYVFFQLELRRVVAG EGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFG FQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVGLFR VTPEIPAGLPSPRSESGREGPELSPDDPAGLLDLRQGMFAQLV AQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKA GVYYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAAL ALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARA RHAWQLTQGATVGLFRVTPEIPAGLPSPRSESGREGPELSPD DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLT GGLSYKEDTKELVVAAGVYYYVFFQLELRRVVAGEGSGSVSL ALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLS SAGQRLGVHLHTEARARHAWQLTQGATVGLFRVTPEIPAGL

	PSPRSEGGSGGSNELHQVPSNCDCLNGGTCVSNKYFSNIHWCN CPKKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPW NSATVLQQTYHAHRSDALQLGLGKHNYCRNPDNRRRPWCYV QVGLKPLVQECMVHDCA (SEQ ID NO: 132)
4-1BB CL:CH1 Fc fusion (Uniprot: 4- 1BB (Q07011 24- 186)): 15AA linker : uPA	LQDPCSNCPAGTFCDNNRNQICSPCPPNSFSSAGGQRTCDICR QCKGVFRTRKECSSTSNAECDCTPGFHCLGAGCSMCEQDCKQ GQELTKKGCKDCCFGTFNDQKRIGICRPWTNCSLDGKSVLVN GTKERDVVCGPSPADLSPGASSVTPPAPAREPGHSPQGGGGSG GGGSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNREGCGGGGSGGGGSGGGGS GGGGSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY ICNVNHKPSNTKVKDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGKGGSG GGGSGGGSGGSNELHQVPSNCDCLNGGTCVSNKYFSNIHW CNCPPKKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPC LPWNSATVLQQTYHAHRSDALQLGLGKHNYCRNPDNRRRP WCYVQVGLKPLVQECMVHDCA (SEQ ID NO: 133)
OX40 CL:CH1 Fc fusion (OX40 Uniprot: P43489 29- 214)): 15AA linker (Variable length linker): uPA (with or without point mutants for mouse uPAR binding)	LHCVGDTYPSNDRCCHECRPGNGMVSRCRSQNTVCRPCGPG FYNDVVSSKPCPKCTWCNLRSGSERKQLCTATQDTCRCRAG TQPLDSYKPGVDCAPCPGHFSPGDNQACKPWTNCTLAGKHT LQPASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPRT SQGPSTRPVEVPGGRRAGGGGSGGGGSGRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN REGCGGGGSGGGGSGGGGSGGGGSGGGSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVKDKRVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALH NHYTQKSLSLSPGKGGSGGGGSGGGGSGGSNELHQVPSNCD LNGGTCVSNKYFSNIHWCNCPPKKFGGQHCEIDKSKTCYEGNG HFYRGKASTDTMGRPCLPWNSATVLQQTYHAHRSDALQLGL GKHN (SEQ ID NO: 134)
GITR CL:CH1 Fc fusion (GITR (Uniprot: Q9Y5U5 26-162)) :	QRPTGGPGCGPGRLLLTGTGTDAARCCRVHTTRCCRDYPGEECCS EWDCCMVQPEFHCGDPCCTTCRHHPCPPGQGVQSQKFSFGF QCIDCASGTFSGGHEGHCKPWTDCQFGFLTVPFGNKTHNAV CVPGSPPAEPGGGGSGGGGSGRTVAAPSVFIFPPSDEQLKSGTAS

15AA linker : human uPA	VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGG GGSGGGGSGGGGSGGGGSGASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLS LSPGKGGSGGGGSGGGGSGGSNELHQVPSNCDCLNGGTCVSN KYFSNIHWCNCPPKFGGQHCCEIDKSKTCYEGNGHFYRGKAST DTMGRPCLPWNSATVLQQTYHAHRSDALQLGLGKH (SEQ ID NO: 135)
scIL- 10_scVIVh1Fc_uPA- ATF - 5aa linker (variable linker length) - uPA-ATF	SPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQ LDNLLLKESLLEDFKGYLGCQALSEMIQFYLEEVMQAENQDP DIKAHVNSLGENLKTLLRLRLRRCHRFLPCENKSKAVEQVKNAF NKLQEKGIYKAMSEFDIFINYIEAYMTMKIRNGGGSPGQGTQS ENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKE SLLEDFKGYLGCQALSEMIQFYLEEVMQAENQDPDIKAHVNS LGENLKTLLRLRLRRCHRFLPCENKSKAVEQVKNAFNKLQEKGI YKAMSEFDIFINYIEAYMTMKIRNGGGGSGGGGSGRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGECGGGGSGGGGSGGGGSGGGGSGASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKR VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGGSFFLYSKLTVDKSRWQQGNVVFSCSV MHEALHNHYTQKSLSLSPGKGGSGGSNELHQVPSNCDCLNGG TCVSNKYFSNIHWCNCPPKFGGQHCCEIDKSKTCYEGNGHFYR GKASTDTMGRPCLPWNSATVLQQTYHAHRSDALQLGLGKH YCRNPDNRRRPWCYVQVGLKPLVQECMVHDCA (SEQ ID NO: 136)
scIL-10_uPA-ATF (variable length linker)-UPA	SPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQ LDNLLLKESLLEDFKGYLGCQALSEMIQFYLEEVMQAENQD PDIKAHVNSLGENLKTLLRLRLRRCHRFLPCENKSKAVEQVKNA AFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRNGGGSPGQGT QSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLL KESLLEDFKGYLGCQALSEMIQFYLEEVMQAENQDPDIKAH VNSLGENLKTLLRLRLRRCHRFLPCENKSKAVEQVKNAFNKLQ EKGIYKAMSEFDIFINYIEAYMTMKIRNGGGSGGGGSGGSNELH QVPSNCDCLNGGTCVSNKYFSNIHWCNCPPKFGGQHCCEIDKS

	KTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQTYHAH RSDALQLGLGKHNYCRNPDRRRPWCVVQVGLKPLVQECM VHDCA SEQ ID NO: (SEQ ID NO: 137)
GM-CSF monomer - huPA-ATF	APARSPSPSTQPWEHVNAIQEARRLLNLSRDTAAEMNETVEVI SEMFDLQEPTCLQTRLELYKQGLRGSLTKLKGPLTMMASHYK QHCPTPETSCATQITFESFKENLKDFLLVIPFDCWEPVQEGGS GGSNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPKKFGGQ HCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQ TYHAHRSDALQLGLGKHNYCRNPDRRRPWCVVQVGLKPLV QECMVHDCA (SEQ ID NO: 138)
IL-12 subunit alpha p35 UniProt: P29459	RNLPVATPDGPMFPCPLHHSQNLLRAVSNMLQKARQTLEFYPC TSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGS CLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMDPK RQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKI KLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO : 139)
IL-12 subunit beta p40 UniProt: P29460	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQ SSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLHKK EDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWTITIST DLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVEC QEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPP KNLQLKPLKNSRQVEVSWEYPTWSTPHSYFSLTFCVQVQGK SKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWA SVPCS (SEQ ID NO: 140)
P40 : 10 AA linker (variable linker length up to 30-40 GlySer): P35 : uPA	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQ SSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLHKK EDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWTITIST DLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVEC QEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPP KNLQLKPLKNSRQVEVSWEYPTWSTPHSYFSLTFCVQVQGK SKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWA SVPCSGSGSGGGSGGRNLPVATPDGPMFPCPLHHSQNLLRAVS NMLQKARQTLEFYPC TSEEIDHEDITKDKTSTVEACLPLELTKN ESCLNSRETSFITNGSCLASRKTSFMMALCLSSIEDLKMYQVE FKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQ KSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASGGGS GGSGGGSSNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCP KKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNS ATVLQQTYHAHRSDALQLGLGKHNYCRNPDRRRPWCVVQV GLKPLVQECMVHDCA (SEQ ID NO: 141)
huPA-ATF : p40 A preferred mutation is the underlined cysteine to serine (C199S)	SNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPKKFGGQH CEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQ TYHAHRSDALQLGLGKHNYCRNPDRRRPWCVVQVGLKPL VQECMVHDCAGGSGGGSGGIWELKKDVYVVELDWYPDAP GEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGD AGQYTCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKT FLRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSDPQGV

	CGAATLSAERVGRDNKEYEYSVEQCQEDSACPAAEESLPIEVM VDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEV SWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSA TVICRKNASISVRAQDRYYSSSWSEWASVPCS (SEQ ID NO: 142)
P35 : huPA- ATF A preferred mutation is the underlined cysteine to serine (C97S)	RNLPVATPDPGMFPCLLHHSQNLLRAVSNMLQKARQTLEFYPC TEEIDHEDITKDKTSTVEACLPLELTKNES <u>CL</u> NSRETSFITNGSC LASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMDPKRQ IFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLC ILLHAFRIRAVTIDRVMSYLNASGGGSGGGSGGGSSNELHQVP SNCDCLNGGTCVSNKYFSNIHWCNCPKKFGGQHCEIDKSKTC YEGNGHFYRGKASTDTMGRPCLPWNSATVLQQTYHAHRSDA LQLGLGKHNYCRNPDRRRPWCVYVQVGLKPLVQECMVHDCA (SEQ ID NO: 143)
> huPA- ATF : P35 : huPA- ATF : A preferred mutation is the underlined cysteine to serine (C97S)	SNELHQVPSNCDCLNGGTCVSNKYFSNIHWCNCPKKFGGQHC EIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQTY HAHRSDALQLGLGKHNYCRNPDRRRPWCVYVQVGLKPLVQE CMVHDCAGGSGGGSGGGRNLPVATPDPGMFPCLLHHSQNLLR AVSNMLQKARQTLEFYPCSEEIDHEDITKDKTSTVEACLPLEL TKNES <u>CL</u> NSRETSFITNGSCLASRKTSFMMALCLSSIEDLKMY QVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSET VPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASG GGSGGGSGGGSSNELHQVPSNCDCLNGGTCVSNKYFSNIHWC NCPKKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLP WNSATVLQQTYHAHRSDALQLGLGKHNYCRNPDRRRPWCVY VQVGLKPLVQECMVHDCAGGSGGHHHHHHGGSGSLNDIFEAQ KIEWHE (SEQ ID NO: 144)
HSA : p40 : uPA A exemplary mutation is the underlined cysteine to serine (C199S)	RGVFRDAAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFE DHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVAT LRETYGEMADCCAKQEPERNECFLQHKDDNPPLRLVRPEVD VMCTAFHDNEETFLKKYL YEIARRHPYFYAPELFFAKRYKAA FTECCQAADKAACLLPKLDELDEGKASSAKQGLKCASLQKF GERAFKAWAVARLSQRFPAEFAEVSKLVTDLT KVHTECCHG DLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAE VENDEMPADLPSLAADFVGSKDVCKNYAEAKDVFLGMFLYE YARRHPDYSVVLRLAKTYETTLEKCCAAADPHECYAKVFD EFKPLVEEPQNLIKQNCLEFEQLGEYKFQNALLVRYTKKVPQV STPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDCLSVFLNQLC VLHEKTPVSDRVTKCCTESLVNGRPCFSALEVDETYVPKEFNA ETFTFHADICTLSEKERQIKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGGS GGGGSGGIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEED GITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSH SLLLHKKEDGIWSTDILKDQKEPKNKTF LRCEAKNYSGRFTC WWLTITISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNK EYEYSVEQCQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFI

	RDIIKPDPPKLNQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTF CVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYY SSSWSEWASVPCSGSGGGSGGSNELHQVPSNCDCLNGGTC VSNKYFSNIHWCNCPKKFGGQHCEIDKSKTCYEGNGHFYRGK ASTDTMGRPCLPWNSATVLQQTYHAHRSDALQLGLGKHNYC RNPDNRRRPWCYVQVGLKPLVQECMVHDCA (SEQ ID NO: 145)
(cpIL-6 GP130 D1)	QNQWLQDMTTHLILRSFKEFLQSSLRALRQMSGGSGGGSSERI DKQIRYILDGISALRKETCNKSNMCESSKEALAENNLNLPKMA EKDGCFSQSGFNEETCLVKIITGLLEFEVYLEYLQNRFESSEEQA RAVQMSTKVLIQFLQKKAKNLDAITTPDPTTNASLLTKLQASE LLDPCGYISPESPVVQLHSNFTAVCVLKEKCMDYFHVNANYIV WKTNHFTIPKEQYTIINRTASSVTFTDIASLNIQLTCNILTFGQLE QNVYGITIISG (SEQ ID NO: 146)
cpIL-6 GP130 D1 : human uPA :	QNQWLQDMTTHLILRSFKEFLQSSLRALRQMSGGSGGGSSERI DKQIRYILDGISALRKETCNKSNMCESSKEALAENNLNLPKM AEKDGCFQSGFNEETCLVKIITGLLEFEVYLEYLQNRFESSEEQ ARAVQMSTKVLIQFLQKKAKNLDAITTPDPTTNASLLTKLQA SELLDPCGYISPESPVVQLHSNFTAVCVLKEKCMDYFHVNAN YIVWKTNHFTIPKEQYTIINRTASSVTFTDIASLNIQLTCNILTF GQLEQNVYGITIISGGGSGGGSGGSNELHQVPSNCDCLNGGT CVSNKYFSNIHWCNCPKKFGGQHCEIDKSKTCYEGNGHFYRG KASTDTMGRPCLPWNSATVLQQTYHAHRSDALQLGLGKHNY CRNPDNRRRPWCYVQVGLKPLVQECMVHDCA (SEQ ID NO: 147)

Recombinant Procedures, Protein Expression and Protein Purification For Example 6

Gene Synthesis and Subcloning into a Mammalian Expression Vector

- 5 Synthesis and subcloning of the genes for expression of the designed constructs was carried out using standard molecular biology methods.

Protein Expression

- 10 All the proteins were expressed using the EXP1293™ Expression System Kit (ThermoFisher Scientific) following the manufacturer's protocol.

Protein Purification

After harvesting the expression culture supernatant, expressed protein was captured depending on the nature of the fusion protein. Proteins containing IgG Fc (mouse or human)

were captured on a protein A column, and the column was washed with up to 5 column volume of PBS. The protein was eluted from the column by lowering the pH of the running buffer and directly neutralized with Tris buffer pH 8. The purified protein was then dialyzed overnight against PBS. For proteins containing a hexahistidine (6xHis) fusion, protein in the supernatant was captured on Ni-NTA sepharose resin and eluted with increasing concentrations of imidazole. The purified protein was then dialyzed overnight against PBS.

Kinetics Measurements of binding of IL-2 and IL-15-based fusion proteins to human or mouse uPAR – Example 6

Recombinant mouse uPAR-Fc (R&D Systems, Cat. No. 531-PA-100) was captured on a BIACORE™ CM5 sensor chip modified using the human antibody capture kit (GE Healthcare, Cat. No. BR100839) as per manufacturer's protocol. The test molecules were passed over the chip at a flow rate of 30 µl/min for 2 minutes in a stepwise manner with an increasing concentration range. Dissociation of analytes was measured for 10 minutes. The chip was regenerated by flowing over 3M MgCl₂ for 30 seconds. The resulting sensorgrams were analyzed with the native instrument software to calculate the binding affinities of the constructs, and the results are presented in **Table 32**.

Recombinant human uPAR his (R&D Systems, 807-UK-100/CF) was biotinylated and then captured on a BIACORE™ Streptavidin sensor chip per manufacturer's protocol. The molecules of interest, also referred to as analytes, were passed over the chip at a flow rate of 30 µl/min for 3 minutes in a stepwise manner with an increasing concentration range. Dissociation of analytes was measured for 20 minutes. The chip was regenerated by flowing over 3M MgCl₂ for 30 seconds. The resulting sensorgrams were analyzed with the native instrument software to calculate the binding affinities of the constructs, and the results are presented in **Table 32**.

Overall, human based IL-2 or IL-15 fusions to uPA-ATF bound tightly to human uPAR, in the range of 0.59-2.2 nM (see **Table 32**, SEQ ID NOs: 98, 99 and 100). These fusion molecules bound with slightly lower potency compared to the control molecule, SEQ ID NO: 20 (human uPA-ATF; 0.24 nM). IL-2 and IL-15 fusions to mouse uPA-ATF bind to mouse uPAR bound with similar potency (0.14-0.24 nM) to SEQ ID NO: 104 (mouse uPA-ATF; 0.14 nM). Additionally, SEQ ID NO: 110, which human cpIL-2:IL-2R α fused to

human uPA-ATF with the N22Y, W30R point mutants incorporate bound tightly to mouse uPAR, with a K_D of 0.12 nM. These data indicate that fusion of IL-2 and IL-15-based sequences to a uPAR targeting moiety, in this case uPA-ATF fused at the N- or C-terminus, retain potent binding to uPAR *in vitro*.

5

Table 32

Construct/SEQ ID NO:	Description	Target	K_D (nM)	k_{on} (1/Ms)	k_d (1/s)
98	Human uPA-ATF:Human cpIL-2:IL-2Ra:His ₆	Human uPAR	2.2	4.6 x 10 ⁵	1.0 x 10 ⁻³
99	Human cpIL-2:IL-2Ra:Human uPA-ATF-His ₆	Human uPAR	0.59	8.3 x 10 ⁵	4.9 x 10 ⁻⁴
100	His ₆ :Human uPA-ATF:hIL-15Ra:cpIL-15	Human uPAR	1.1	5.0 x 10 ⁵	5.6 x 10 ⁻⁴
101	Mouse cpIL-2:IL-2Ra:mouse uPA-ATF-His ₆	Mouse uPAR	0.19	1.3 x 10 ⁶	2.6 x 10 ⁻⁴
102	His ₆ :Mouse uPA-ATF:mouse IL-15Ra:cpIL-15	Mouse uPAR	0.14	1.5 x 10 ⁶	2.0 x 10 ⁻⁴
103	Human uPA-ATF:His ₆	Human uPAR	0.24	1.5 x 10 ⁶	3.5 x 10 ⁻⁴
104	Mouse uPA-ATF:His ₆	Mouse uPAR	0.15	2.2 x 10 ⁶	3.2 x 10 ⁻⁴
105	Mouse uPA-ATF:Mouse cpIL-2:IL-2Ra:His ₆	Mouse uPAR	0.14	1.4 x 10 ⁶	2.0 x 10 ⁻⁴
110	Human cpIL-2:IL-2Rα:Human uPA-ATF (N22Y, W30R)-His ₆	Mouse uPAR	0.12	1.9 x 10 ⁶	2.3 x 10 ⁻⁴

In vitro potency of molecules designed to activate the IL-2 Receptor on human T cells –

Example 6

In order to assess the potency of molecules containing human sequence-based fusions of IL-2 or IL-15, HH cells (ATCC® CRL-2105™) were incubated for 30 minutes with increasing concentrations of SEQ ID NOs: 98, 99, 100, 110, 94, 106, and 107. After

incubation, cells were washed and lysed, and phosphorylation of STAT5 was detected using the STAT5 alpha/beta (Phospho) [pY694/pY699] Multispecies InstantOne™ ELISA Kit (ThermoFisher Scientific, Cat. No. 85-86112-11) as per the manufacturer's protocol. Phosphorylation of STAT5 is a receptor-proximal signaling event induced upon IL-2/IL-15 receptor activation. The data is shown in **Table 33**.

The non-targeted, parental circularly permuted cpIL-2:IL2R α fusion protein, SEQ ID NO: 94, exhibited an EC₅₀ of 1.1 nM in the HH pSTAT5 assay. cpIL-2:IL2 R α fusion proteins with a C-terminal uPAR targeting domain, i.e. SEQ ID NOs: 99, 106 and 110 were similarly potent, with EC₅₀s of 1.5 nM, 1.6 nM, and 2.1 nM, respectively. SEQ ID NO: 98, a cpIL-2:IL2 R α fusion protein with an N-terminal uPAR targeting domain was less potent with an EC₅₀ of 7-10 nM. The non-targeted, parental hIL-15Ra(sushi): cpIL-15 fusion protein, SEQ ID NO: 95 is more potent than SEQ ID NO: 94 with an EC₅₀ of 0.039 nM in the HH pSTAT5 assay. Variants of SEQ ID NO: 95 with an N-terminal targeting domain were also more potent than SEQ ID NO: 94, with EC₅₀s of 0.18 nM and 0.099 nM for SEQ ID NOs: 100 and 107 respectively. In summary, targeted variants of SEQ ID NO: 94 with a C-terminal uPAR targeting domain and targeted variants of SEQ ID NO: 95 with an N-terminal targeting domain were all equipotent with their non-targeted parental molecules, suggesting that fusion to the uPAR targeting motif does not sterically hinder/interfere with the cytokine domains binding to their cognate receptor.

Table 33

Construct/SEQ ID NO:	Description	pSTAT5 EC ₅₀ (nM)
94	Human cpIL-2:IL-2R α	1.1
95	Human IL-15Ra:cpIL-15	0.039
98	Human uPA-ATF:Human cpIL-2:IL-2R α :His ₆	7-10
99	Human cpIL-2:IL-2R α :Human uPA-ATF-His ₆	1.5
100	His ₆ :Human uPA-ATF:hIL-15Ra:cpIL-15	0.18
110	Human cpIL-2:IL-2R α :Human uPA-ATF (N22Y, W30R)-His ₆	1.6

106	Human cpIL-2:IL-2Ra:Fc:Human uPA-ATF	2.1
107	Human uPA-ATF:Fc:hIL-15Ra:cpIL-15	0.099

In vitro potency of molecules designed to activate the IL-2 Receptor on primary mouse splenocytes – Example 6

In order to query the potency of molecules containing mouse sequence-based fusions of IL-2 or IL-15, splenocytes were harvested from the spleens of wild-type mice, incubated for 30 minutes with increasing concentrations of SEQ ID NO: 96, a mouse Fc: mouse IL-15Ra:cpIL-15, SEQ ID NO: 101, SEQ ID NO: 102 and SEQ ID NO: 105. Cells were then fixed and stained with fluorescently-labeled antibodies against surface and intracellular markers to detect phosphorylation of STAT5 on mouse NK cells, CD8 T cells and CD4+CD25⁺ Foxp3⁺ regulatory T cells (T_{regs}) using standard flow cytometry techniques. The results are shown in **Table 34**.

Recombinant wild-type IL-2 (rIL-2) selectively stimulates cells that express the high affinity IL-2 receptor, which in normal mice and healthy humans is expressed mostly by T_{regs}. At much higher concentrations, rIL-2 can also activate NK cells and subsets of memory CD8 T cells due to their expression of the intermediate affinity IL-2 receptor complex. The circularly permuted mouse cpIL-2:IL2R α fusion protein, SEQ ID NO: 96, was engineered to be more potent on cells expressing the intermediate affinity receptor relative to rIL-2. This is demonstrated by the equipotent induction of pSTAT5 in T_{regs}, NK cells, and memory CD8 T cells with EC₅₀s of 21.6 nM, 19.0 nM, and 23.2 nM, respectively. A mouse cpIL-2:IL2 R α fusion protein with a C-terminal uPAR targeting domain, i.e. SEQ ID NO: 101 was similarly potent on T_{regs}, NK cells, and memory CD8 T cells with EC₅₀s of 16.5 nM, 19.5 nM, and 27.3 nM, respectively. Similarly, a mouse cpIL-2:IL2 R α fusion protein with an N-terminal uPAR targeting domain, i.e. SEQ ID NO: 105, exhibited EC₅₀s of 21.9 nM, 34.9 nM, and 38.0 nM on T_{regs}, NK cells, and memory CD8 T cells, respectively. In contrast to rIL-2, rmIL-15 does not exhibit selective activation for T_{regs} as its affinity for the IL-2/IL-15 receptor complex is not enhanced by the presence of CD25. This is demonstrated by the similar induction of pSTAT5 in T_{regs}, NK cells, and memory CD8 T cells by the mouse Fc: mouse IL-15Ra:cpIL-15 with EC₅₀s of 0.60 nM, 0.047 nM, and 0.15 nM, respectively. A mouse IL-

15Ra(sushi):cpIL-15 fusion protein with an N-terminal uPAR targeting domain, i.e. SEQ ID NO: 102, exhibited similar potency on T_{regs}, NK cells, and memory CD8 T cells with EC₅₀s of 1.7 nM, 0.15 nM, and 0.50 nM, respectively. As is the case for uPAR-targeted variants of SEQ ID NO: 94 and SEQ ID NO: 95, uPAR-targeted variants of the murine orthologs were all equipotent with their non-targeted parental molecules, suggesting that fusion to the uPAR targeting motif does not sterically hinder/interfere with the cytokine domains binding to their cognate receptor expressed on primary mouse T_{regs}, NK cells and memory CD8 T cells.

Table 34

Construct/SEQ ID NO:	Description	T _{regs} EC ₅₀ (nM)	NK Cell EC ₅₀ (nM)	Memory CD8 T Cell EC ₅₀ (nM)
96	Mouse cpIL-2:IL-2Ra	22	19	23
xx	mouse Fc:IL-15Ra:cpIL-15	0.60	0.047	0.15
101	Mouse cpIL-2:IL-2Ra:mouse uPA-ATF-His ₆	17	20	27
102	His ₆ :Mouse uPA-ATF:mouse IL-15Ra:cpIL-15	1.7	0.15	0.50
105	Mouse uPA-ATF:Mouse cpIL-2:IL-2Ra:His ₆	22	35	38

10

Binding of IL-2 or IL-15-based molecules to mouse tumor cells lines that express uPAR
– Example 6

15 Mouse syngeneic tumor lines 4T1, CT26, EMT6 (former all obtained from ATCC®) and MC38 (from NCI) were shown to express mouse uPAR on the cell surface by staining with a fluorescently labeled anti-mouse uPAR (CD87) antibody, as detected by flow

cytometry. Adherent cells were lifted with CORNING® CELLSTRIPPER® Solution (Cat. No. 25056CI), washed, and then incubated with increasing concentrations of test molecules (see **Table 35**). After incubation at 4°C for 60 min, cells were stained with a fluorescently-labeled anti-His antibody and analyzed using standard flow cytometry techniques. Mean fluorescence intensity were plotted against test article concentration and EC₅₀ values were calculated using a 4-parameter logistic curve fit.

Table 35

Tumor Line	4T1 EC ₅₀ (nM)	CT26 EC ₅₀ (nM)	EMT6 EC ₅₀ (nM)	MC38 EC ₅₀ (nM)
SEQ ID NO: 101	1.0	1.3	0.41	0.71
SEQ ID NO: 102	ND	ND	ND	0.41
SEQ ID NO: 103	0.22	1.1	ND	0.42
SEQ ID NO: 110	ND	1.4	0.54	ND

10 Pharmacokinetics in MC38 Tumor-Bearing Mice of SEQ ID NO: 94 and SEQ ID NO: 110 – Example 6

In order to determine the ratio of protein circulating in serum relative to tumors, a single dose PK experiment was carried out in MC38 tumor-bearing female C57BL/6 mice. Mice were dosed when tumors size ranged from 200-400 mm³. Mice were dosed subcutaneously with either 30 µg of SEQ ID NO: 94 or 75 µg SEQ ID NO: 110 at time 0, and then terminal bleeds were collected and processed to serum for each individual animal at 0.5, 2, 4, 8, and 24 hours post dose. Tumors from the same animals were also harvested and snap frozen for downstream concentration analysis. Serum samples were analyzed by a human IL-2-specific MSD (Meso-Scale Discovery) assay. Tumor samples were homogenized and concentrations measured by the same detection assay. Pharmacokinetic parameters were

determined using a non-compartmental model in WinNonlin (Phoenix). The results are shown in **Table 36**.

When comparing the ratio of tumor exposure to systemic exposure (T/S) for both C_{max} and AUC, SEQ ID NO: 110, a uPAR-targeted IL-2, showed a greater ratio of tumor penetration compared to the non-targeted control molecule SEQ ID NO: 94, which showed a similar ratio of exposure in the tumor relative to serum.

Table 36

Construct/ SEQ ID NO:	Matrix	C _{max} (nM)	T _{max} (h)	AUC _{last} (h*nM)	AUC _{inf} (h*nM)	MRT _{inf} (h)	t _{1/2} (h)	T/S (C _{max}) (ratio)	T/S (AUC _{inf}) (ratio)
94	Serum	19.5	2	234	239	9.9	8.3	NA	NA
94	Tumor	23.2	2	189	191	6.6	8	1.2	0.8
110	Serum	2.9	4	29	29.1	7.3	6.3	NA	NA
110	Tumor	31.3	2	155	157	4.2	5.9	10.8	5.4

In vitro potency of molecules designed to activate the IL-10 receptor – Example 6

STAT3 phosphorylation is a receptor-proximal signaling event in response to binding and activating the IL-10 receptor. In order to query the potency of molecules containing fusions of IL-10, human peripheral blood mononuclear cells were incubated for 1 hour with increasing concentrations of scIL-10:CL:CH1:Fc (scIL10 3aa linker); = SEQ ID NO: 136 which is equivalent to: scIL-10:CL:CH1:Fc plus 5AA linker plus huPA-ATF). Cells were then fixed and stained with fluorescently-labeled antibodies against surface and intracellular markers to detect phosphorylation of STAT3 on mouse B cells, monocytes, CD4 and CD8 T cells using standard flow cytometry techniques. The data is shown in **Table 37**.

Both molecules have very similar potency to each other across various cell types, supporting that fusion of IL-10 molecules to a uPAR targeting moiety, in this case the human uPA-ATF, does not affect the ability of the molecules to bind and activate the IL-10 receptor expressed on human immune cells.

Table 37

Cell population	scIL-10:CL:CH1:Fc (scIL10 3aa linker)	SEQ ID NO: 136
CD8 Central Memory T cells	91.0 pM	49.9 pM
CD4 Central Memory T cells	80.6 pM	111 pM
Monocytes	0.060 pM	0.037 pM
B cells	0.057 pM	0.127 pM

In vitro potency of uPAR-targeted molecules designed to activate the IL-12 receptor –**Example 6**

5 HEK-BLUE™ IL-12 cells (InvivoGen) are human embryonic kidney cells specifically designed to detect bioactive IL-12 in vitro by monitoring the IL-12-induced activation of the NF-κB/AP-1 pathways. The cell line expresses an inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene under control of the STAT4-inducible promoter. HEK-Blue IL-1β cells were plated at 50,000 cells/well in DMEM media containing 4.5 g/L glucose and 2 mM L-glu and 10% heat inactivated FBS (Gibco). Cells were incubated for 20 hours at 37° C, 5% CO₂ with varying concentrations of IL-12 (R&D Systems, Cat. No. 219-IL-005/CF) or SEQ ID NO: 141. SEAP production was detected by adding QUANTI-Blue™ (InvivoGen) and incubating for 3 hours at 37° C, 5% CO₂ and then read on a plate reader at 630 nm.

15 The single chain human IL-12 construct fused to the human uPA-ATF construct of SEQ ID NO: 141 has similar potency (27 pM) compared to wild-type human IL-12 (18 pM) suggesting that fusion of IL-12 to uPA-ATF does not affect the ability of the IL-12 portion of the molecules to bind and activate the IL-12 receptor.

20 **In vitro potency of uPAR-targeted molecules designed to active the human 4-1BB receptor – Example 6**

Fusion proteins containing 4-1BB ligand were assayed using the 4-1BB Bioassay Kit from Promega. The assay uses 4-1BB effector cells that stably express 4-1BB and luciferase

downstream of a response element. Luciferase expression is induced upon ligand binding to the receptor. The assay was performed per manufacturer's protocol. Dose response curves were generated by incubating test molecules at increasing concentrations and measuring generated light from expressed luciferase. The data is shown in **Table 38**.

5 Recombinant human 4-1BB ligand monomer (R&D Systems Cat. No. 2295-4L-025/CF) displayed no activity in the assay, suggesting that uncrosslinked 4-1BBL monomer is not able to activate 4-1BB, as 4-1BBL normally functions as a trimer. Linked 4-1BBL trimers fused to human uPA-ATF at the N- or C-terminus (SEQ ID NO: 130 and SEQ ID NO: 131) have more a more potent EC_{50} (and higher maximal signal compared to a linked 4-1BBL trimer molecule that is not fused to the human uPA-ATF (SEQ ID NO: 129), which has an EC_{50} of 0.15 nM. These results suggest that fusion to the human uPAR-targeting domain uPA-ATF may result in 2-3 fold more potent activity in signaling through 4-1BB, potentially due to avidity affects due to potent binding to uPAR on the cell surface. Nonetheless, fusion proteins of 4-1BBL to uPA-ATF are functionally able to bind and signal through 4-1BB.

15

Table 38

Test molecule	description	EC_{50} (nM)	E _{max} (luciferase units)
4-1BBL monomer	Control (from R&D systems)	No activity	No activity
SEQ ID NO: 129	linked human 4-1BBL Trimer	0.15	193,000
SEQ ID NO: 130	Human uPA-ATF: linked human 4-1BBL Trimer	0.046	266,000
SEQ ID NO: 131	Linked human 4-1BBL Trimer: Human uPA-ATF	0.10	330,000

20

In vitro potency of molecules designed to activate the GM-CSF receptor on human monocytes – Example 6

In order to assess the potency of molecules containing human sequence-based fusions of GM-CSF, U937 cells (ATCC® CRL-1593.2™) were incubated for 30 minutes with increasing concentrations of recombinant human GM-CSF (R&D Systems, cat # 215-GM-050/CF), SEQ ID NO: 138 or SEQ ID NO: 103. After incubation, cells were fixed, permeabilized and stained intracellularly with a fluorescently-labeled antibody specific for pSTAT5 and analyzed using standard flow cytometry techniques. The data is shown in **Table 39**.

Recombinant human GM-CSF (rhGM-CSF) is a potent inducer of STAT5 phosphorylation as demonstrated by an EC₅₀ of 18.6 pM in the U937 pSTAT5 assay. A human uPAR-targeting domain was fused at the C-terminal end of GM-CSF in SEQ ID NO: 138. SEQ ID NO: 138 was approximately 20-fold more potent in inducing STAT5 phosphorylation, with an EC₅₀ of 1.28 pM. Co-incubation of SEQ ID NO: 138 with increasing concentrations of the human uPAR-targeting domain alone, SEQ ID NO: 103, resulted in decreased potency, similar to rhGM-CSF alone. These results suggest that fusion of the uPAR-targeting motif to GM-CSF did not negatively impact the ability of the fusion protein to induce STAT5 phosphorylation as a result of receptor binding, rather it appears to enhance its activity.

Table 39

Test molecule	Competitor	EC ₅₀ (pM)
rhGM-CSF	none	18.6
SEQ ID NO: 138	none	1.28
SEQ ID NO: 138	1 nM human uPA-ATF	2.55
SEQ ID NO: 138	100 nM human uPA-ATF	14.0

In Vivo Pharmacokinetics of Exemplary Fusion Proteins

Select human anti-uPAR – IL-12 subunit fusion proteins were analyzed *in vivo* in mouse tumor models. Specifically, levels of the tested fusion proteins were detected in serum and tumor tissue of mice at several timepoints post administration. The fusion proteins used in this section are recited below in **Table 40**. Experimental details are outlined below in **Table 41**.

Table 40 – Amino Acid Sequences of Fusion Proteins

Description	Amino Acid Sequence/SEQ ID NO
p35-hM1- ScFv(vH3vL4)2	RNLPVATPDPMFPCLLHHSQNLLRAVDNMLQKARQTLEFYF CTSEEDHEDITKDKTSTVEACLPLELTKNESKLNSRETSFITN GSCLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMD PKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKT KIKLCILLHAFRIRAVTINRVMSYLNASGGGGSGGGGSEVQLQ QSGAEVKKPGATVKLSCTASGFNIKDEYINWVKQRPQGGL WIGWIDPENGDEYASKFQGRATITADTSTDYAYLELSSLRSE DTAVYYCTGGNYVGFYWGQGLTVTVSSGGGGSGGGGSG GGGSQIVLTQSPATLSASPGERVMTSCSASSSVSYMHYQQK PGTSPRRWLYDTSKLASGVPARFSGSGSGTDYTLTISSLEPEDF ATYYCQQWSSNPPYTFGQGTKLEIKGGSGGGSGGGSGGGG SGGGSGGGSGGEVQLQQSGAEVKKPGATVKLSCTASGFNIK DEYINWVKQRPQGGLWIGWIDPENGDEYASKFQGRATITA DTSTDYAYLELSSLRSEDTAVYYCTGGNYVGFYWGQGLTV TVSSGGGGSGGGSGGGGSQIVLTQSPATLSASPGERVMTS CSASSSVSYMHYQQKPGTSPRRWLYDTSKLASGVPARFSGS GSGTDYTLTISSLEPEDFATYYCQQWSSNPPYTFGQGTKLEIK (SEQ ID NO: 189)
p35-hM8- ScFv(vB10vH3)2	RNLPVATPDPMFPCLLHHSQNLLRAVDNMLQKARQTLEFYF CTSEEDHEDITKDKTSTVEACLPLELTKNESKLNSRETSFITN GSCLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMD PKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKT KIKLCILLHAFRIRAVTINRVMSYLNASGGGGSGGGGSDIQMT QSPSSLSASVGDVTITCRTSENIYSNLAWYQQKPGKSPQLLV YAATNLADGVPSRFSGSGSGTDYTLTISSLQPEDFGTYQCQHF WGTPWTFGGGTVKVEIKGGGGSGGGSGGGGSQIQLVQSGSE LKKPGASVKISCKASGTTFTDYGMNWVKQSPGKGLKWMGW INTNTGEPTYAEDFKGRFVFLDTSVSTAYLQISSLKAEDTAT YFCAHYSFDYWGGQGLTVTVSSGGSGGGSGGGSGGGSGGG GGGGSGGGDIQMTQSPSSLSASVGDVTITCRTSENIYSNLAW YQQKPGKSPQLLVYAATNLADGVPSRFSGSGSGTDYTLTISSL QPEDFGTYQCQHFWGTPWTFGGGTVKVEIKGGGGSGGGSGGG GGSQIQLVQSGSELKKPGASVKISCKASGTTFTDYGMNWVKQ

	SPGKGLKWMGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYL QISSLKAEDTATYFCAHYSFDYWGQGTLTVSS (SEQ ID NO: 213)
hM1-IgG1-p40 Heavy Chain (hM1-IgG1 (H3)-p40 (HC-fusion))	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYINWVKQRP QGLEWIGWIDPENGDTHEYASKFQGRATITADTSTDYAYLELSS LRSEDTAVYYCTGGNYVGWFPYWGQGLTVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSIWEL KKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLQDQSS VLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHLLLLHKKED GIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWTITSTD LTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVEC QEDSASPAAEESLPIEVMVDAVHKLKYENYTSFFIRDIKPD PKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQ KSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSE WASVPCS (SEQ ID NO: 178)
hM1-IgG1-p40 Light Chain (hM1-L3 (Kappa LC))	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWYQQKPGTSP RRWIYDTSKLASGVPARFSGSGSGTDFTLTISSELPEDFAVYY CQQWSSNPPYTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C (SEQ ID NO: 159)
hM8-IgG1-p40 Heavy Chain (hM8-IgG1(H3)-p40 (HC-fusion))	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGMNWVKQSPG KGLKWMGWINTNTGEPTYAEDFKGRFVFSLDTSVSTAYLQIS SLKAEDTATYFCAHYSFDYWGQGTLTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSIWELKK DVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLQDQSS EVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHLLLLHKKED GIWSTDILKDQKEPKNKTFRLCEAKNYSGRFTCWWTITSTD LTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVEC QEDSASPAAEESLPIEVMVDAVHKLKYENYTSFFIRDIKPD PKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFSVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSE WASVPCS

	CS (SEQ ID NO: 193)
hM8-IgG1-p40 Light Chain (hM8-B2 (Kappa LC))	DIQMTQSPSSLSASVGDVTITCRTSENIYSNLAWYQQKPGKS PQLLVYAATNLADGVPSRFSGSGSGTQYTLKISSLPEDFATY YCQHFHWGTPWTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C (SEQ ID NO: 161)
hM1-Fab-p40 Heavy Chain (hM1-Fab(H3)-p40 (HC-fusion))	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYNWVKQRPG QGLEWIGWIDPENGDTHEYASKFQGRATITADTSTDYAYLELSS LRSEDTAVYYCTGGNYVGFYWGQGLTVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCGGGSGGGGSIWELKKDYYVVELDWYPDAP GEMVVLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVKEFGD AGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNK TFLRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSDPQGV CGAATLSAERVRGDNKEYEYSVEQCEDSASPAAEESLPIEVM VDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEV SWEYPDTWSTPHSYFSLTFSVQVQGKSKREKKDRVFTDKTSA TVICRKNASISVRAQDRYYSSSWSEWASVPCS (SEQ ID NO: 179)
hM1-Fab-p40 Light Chain (hM1-L3 (Kappa LC))	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWYQQKPGTSP RRWIYDTSKLASGVPARFSGSGSGTDFTLTISSELPEDFAVYY CQQWSSNPPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C (SEQ ID NO: 159)
hM8-Fab-p40 Heavy Chain (hM8-Fab(H3)-p40 (HC fusion))	QIQLVQSGSELKKPGASVKISCKASGTTFTDYGMNWVKQSPG KGLKWMGWINTNTGEPTYAEDFKGRFVFLDTSVSTAYLQIS SLKAEDTATYFCAHYSFDYWGGQGLTVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCGGGSGGGGSIWELKKDYYVVELDWYPDAPGEMV VLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNK TFLRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSDPQGV TCGAATLSAERVRGDNKEYEYSVEQCEDSASPAAEESLPIEVM VDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEV SWEYPDTWSTPHSYFSLTFSVQVQGKSKREKKDRVFTDKTSA TVICRKNASISVRAQDRYYSSSWSEWASVPCS (SEQ ID NO: 194)
hM8-Fab-p40 Light Chain (hM8-B5 (Kappa LC))	DIQMTQSPSSLSASVGDRTITCRTSENIYSNLAWYQQKPGKS PQLLVYAATNLADGVPSRFSGSGSGTDYTLTISSELPEDFGTY YCQHFHWGTPWTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C (SEQ ID NO: 162)

p35-hM1-Fab-KiH Heavy Chain (hM1-Fab(H3)-KiH (knob))	EVQLQQSGAEVKKPGATVKLSCTASGFNIKDEYINWVKQRP QGLEWIGWIDPENGDT EYASKFQGRATITADTSTD TAYLELSS LRSEDTAVYYCTGGNYVGWFPYWGQGT LVT VSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMI SRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKT ISKAKGQPREPQVCTLPSSREEMTKNQVSLWCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 238)
p35-hM1-Fab-KiH Light Chain (hM1-L3 (Kappa LC))	EIVLTQSPATLSLSPGERATLSCSASSSVSYMHYQKPGTSP RRWIYDTSKLASGVPARFSGSGSGTDFTLTISSELPEDFAVYY CQQWSSNPPYTFGQGT KLEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C (SEQ ID NO: 159)
p35-hM1-Fab-KiH (p35-Fab-KiH (hole))	RNLPVATPDPMFPCLHHSQNLLRAVDNMLQKARQTLEFYF CTSEEIDHEDITKDKTSTVEACLPLELTKNESKLNSRETSFITN GSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMD PKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEDFYKT KIKLCILLHAFRIRAVTINRVMSYLNASGGGSGGGSGGEPKSS DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV VLT V LHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREP QVYTLPPCREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNRFTQKSLSLSPGK (SEQ ID NO: 239)

Table 41 – Assay Details

Group	Number of animals	Test Article	Structure	Dose Route	Target Dose Level (mg/kg)	Detection Assay
1	6	vehicle				
2	36	SEQ ID NO: 213	p35-hM8- ScFv2	bolus	40	MSD anti-human P35 assay
3	36	SEQ ID NO: 213	p35-hM8- ScFv2	infusion	40	MSD anti-human P35 assay
4	36	SEQ ID NO: 189	p35-hM1- ScFv2	bolus	40	MSD anti-human P35 assay
5	36	SEQ ID NO: 179 & SEQ ID NO: 159	hM1-Fab- P40	bolus	6.9	MSD Human IL- 12p40 V-Plex Kit
6	36	SEQ ID NO: 213 (0.5h prior to P40)	p35-hM8- ScFv2	infusion	40	MSD Human IL- 12p70 V-Plex Kit

		SEQ ID NO: 178 & SEQ ID NO: 159	hM1-IgG- P40	bolus	11.7	
7	36	SEQ ID NO: 213 (4h prior to P40)	p35-hM8- ScFv2	infusion	40	MSD Human IL- 12p70 V-Plex Kit
		SEQ ID NO: 178 & SEQ ID NO: 159	hM1-IgG- P40	bolus	11.7	

The model used in the above study was the SC RKO human colorectum cancer xenograft model in female NOD/SCID mice. To generate the tumor model, each mouse was inoculated subcutaneously with RKO tumor cells. Randomization was used when tumor size reached $\sim 250 \text{ mm}^3$. Tumor and serum samples were taken at 0.5, 1, 4, 24, 48, 72 hours post last dose. The Meso Scale Discovery (MSD) assays were used for the detection of p35, p40, or p70 in the samples accordingly to manufacture protocols.

As shown in **Fig. 11**, the tested fusion proteins in each Group were detected in tumor tissue at all timepoints testes, with levels remaining relatively stable. The data shows that the fusion proteins productively accumulate in tumor tissue. As shown in **Fig. 12**, for select Groups, the level of the fusion proteins in serum steadily dropped over time, demonstrating that the fusion protein is rapidly cleared from the system except for fusion protein in the target tumor tissue.

CLAIMS

What is claimed is:

1. An isolated antigen binding protein that binds specifically to urokinase plasminogen
 5 activator receptor (uPAR), comprising:
 - (a) an antibody heavy chain variable (VH) domain comprising:
 - i) an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), GFSLTNYG (SEQ ID NO: 11), GNTFTDYG (SEQ ID NO: 19), GTTFTDYG (SEQ ID NO: 41), or GYTFTSYG (SEQ ID NO: 27);
 - 10 ii) an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), IWSDGGT (SEQ ID NO: 12), INTNTGEP (SEQ ID NO: 20), or IYPRSGNT (SEQ ID NO: 28); and
 - iii) an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5), ARGGRSDLFAY (SEQ ID NO: 13), AHYSFDY (SEQ ID NO: 21), or AGKDYGSTYADY (SEQ ID NO: 29); and
 - 15 (b) an antibody light chain variable (VL) domain comprising:
 - iv) an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), QSIVHSNGNTY (SEQ ID NO: 14), ENIYSN (SEQ ID NO: 22), SSVSSRY (SEQ ID NO: 30);
 - v) an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), KVS (SEQ ID NO: 15), AAT (SEQ ID NO: 23), or GTS (SEQ ID NO: 31); and
 - 20 vi) an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8), FQGSHVPYT (SEQ ID NO: 16), QHFWGTPWT (SEQ ID NO: 24), QQYHSDPLT (SEQ ID NO: 32).
2. The antigen binding protein of claim 1, wherein the VH domain comprises an amino
 25 acid sequence of SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 37, SEQ ID NO: 42, or SEQ ID NO: 43, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, or SEQ ID NO: 44.
3. The antigen binding protein of claim 1, wherein:

- a) the VH domain comprises an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5); and
- b) the VL domain comprises an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), and an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8).
4. The antigen binding protein of claim 1, wherein:
- a) the VH domain comprises an HCDR1 amino acid sequence of GFSLTNYG (SEQ ID NO: 11), an HCDR2 amino acid sequence of IWSDGGT (SEQ ID NO: 12), an HCDR3 amino acid sequence of ARGGRSDLFAY (SEQ ID NO: 13); and
- b) the VL domain comprises an LCDR1 amino acid sequence of QSIVHSNGNTY (SEQ ID NO: 14), an LCDR2 amino acid sequence of KVS (SEQ ID NO: 15), and an LCDR3 amino acid sequence of FQGSHVPYT (SEQ ID NO: 16).
5. The antigen binding protein of claim 1, wherein:
- a) the VH domain comprises an HCDR1 amino acid sequence of GNTFTDYG (SEQ ID NO: 19), or GTTFTDYG (SEQ ID NO: 41), an HCDR2 amino acid sequence of INTNTGEP (SEQ ID NO: 20), an HCDR3 amino acid sequence of AHYSFDY (SEQ ID NO: 21); and
- b) the VL domain comprises an LCDR1 amino acid sequence of ENIYSN (SEQ ID NO: 22), an LCDR2 amino acid sequence of AAT (SEQ ID NO: 23), and an LCDR3 amino acid sequence of QHFWGTPWT (SEQ ID NO: 24).
6. The antigen binding protein of claim 1, wherein:
- a) the VH domain comprises an HCDR1 amino acid sequence of GYTFTSYG (SEQ ID NO: 27), an HCDR2 amino acid sequence of IYPRSGNT (SEQ ID NO: 28), an HCDR3 amino acid sequence of AGKDYGSTYADY (SEQ ID NO: 29); and
- b) the VL domain comprises an LCDR1 amino acid sequence of SSVSSRY (SEQ ID NO: 30), an LCDR2 amino acid sequence of GTS (SEQ ID NO: 31), and an LCDR3 amino acid sequence of QQYHSDPLT (SEQ ID NO: 32).

7. The antigen binding protein of any one of claims 1-6, wherein:
- a) the VH domain comprises an amino acid sequence of SEQ ID NO: 33, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35;
 - 5 b) the VH domain comprises an amino acid sequence of SEQ ID NO: 33, and the VL domain comprises an amino acid sequence of SEQ ID NO: 36;
 - c) the VH domain comprises an amino acid sequence of SEQ ID NO: 34, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35;
 - d) the VH domain comprises an amino acid sequence of SEQ ID NO: 34, and the VL domain comprises an amino acid sequence of SEQ ID NO: 36;
 - 10 e) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 38;
 - f) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 39;
 - 15 g) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 40;
 - h) the VH domain comprises an amino acid sequence of SEQ ID NO: 42, and the VL domain comprises an amino acid sequence of SEQ ID NO: 44; or
 - i) the VH domain comprises an amino acid sequence of SEQ ID NO: 43, and the VL domain comprises an amino acid sequence of SEQ ID NO: 44.
 - 20
8. The antigen binding protein of claim 7, wherein the VH domain and VL domain are attached with an amino acid linker.
- 25 9. The antigen binding protein of claim 8, wherein the amino acid linker comprises (GGGGS)_n (SEQ ID NO: 148), wherein n is an integer between 1 and 5.
10. The antigen binding protein of claim 8, wherein the amino acid linker comprises the amino acid sequence GGGSGGGSGGGGS (SEQ ID NO: 149),
- 30 GGS GGGSGGGSGGGSGGGSGGGSGG (SEQ ID NO: 150), or GGGSGGGSGG (SEQ ID NO: 246).

11. The antigen binding protein of any one of claims 1-10, wherein the antigen binding protein comprises a Fab fragment, a F(ab')₂ fragment, an Fd fragment, an Fv fragment, a single chain Fv (scFv), a dAb fragment, a single domain antibody, or a nanobody.
- 5 12. The antigen binding protein of any one of claims 1-10, comprising the amino acid sequence of any one of SEQ ID NOs: 56-71.
- 10 13. The antigen binding protein of any one of claims 1-12, further comprising the amino acid sequence of any one of SEQ ID NOs: 72-78.
14. The antigen binding protein of any one of claims 1-13, comprising an antibody heavy chain (HC) amino acid sequence of SEQ ID NO: 45, 46, 49, 53, or 54, and an antibody light chain (LC) amino acid sequence of SEQ ID NO: 47, 48, 50, 51, 52, and 55.
- 15 15. The antigen binding protein of claim 14, wherein:
- a) the antibody HC comprises the amino acid sequence of SEQ ID NO: 45, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 47;
- b) the antibody HC comprises the amino acid sequence of SEQ ID NO: 45, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 48;
- 20 c) the antibody HC comprises the amino acid sequence of SEQ ID NO: 46, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 47;
- d) the antibody HC comprises the amino acid sequence of SEQ ID NO: 46, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 48;
- 25 e) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 50;
- f) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 51;
- g) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 52;
- 30

h) the antibody HC comprises the amino acid sequence of SEQ ID NO: 53, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 55; or

i) the antibody HC comprises the amino acid sequence of SEQ ID NO: 54, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 55.

5

16. The antigen binding protein of any one of claims 1-10, wherein the antigen binding protein comprises an amino acid sequence of SEQ ID NO: 189.

17. The antigen binding protein of any one of claims 1-10, wherein the antigen binding
10 protein comprises an amino acid sequence of SEQ ID NO: 213.

18. The antigen binding protein of any one of claims 1-10, comprising:
a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 178; and
a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159.

15

19. The antigen binding protein of any one of claims 1-10, comprising:
a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 193; and
a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 161.

20. 20. The antigen binding protein of any one of claims 1-10, comprising:
a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 179; and
a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159.

21. The antigen binding protein of any one of claims 1-10, comprising:
25 a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 194; and
a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 162.

22. The antigen binding protein of any one of claims 1-10, comprising:
a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 238;
30 a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159;

and

a third polypeptide chain comprising an amino acid sequence of SEQ ID NO: 239.

23. The antigen binding protein of any one of claims 1-22, wherein the antigen binding
5 protein binds human uPAR with a KD of less than about 5×10^{-8} M, less than about 1×10^{-8}
M, less than about 5×10^{-9} M, less than about 1×10^{-9} M, less than about 5×10^{-10} M, or less
than about 1×10^{-10} M.
24. The antigen binding protein of any one of claims 1-23, wherein the antigen binding
10 protein binds human uPAR in the presence of a uPAR ligand.
25. The antigen binding protein of any one of claims 1-23, wherein the antigen binding
protein thereof binds uPAR ligand-bound human uPAR.
- 15 26. The antigen binding protein of any one of claims 1-23, wherein the antigen binding
protein binds human uPAR on human uPAR-expressing cells, thereby producing a fluorescent
signal detected with a fluorescently-labelled antibody that binds to the antigen binding
protein, with an EC50 value of about 0.1 nM to about 3.0 nM.
- 20 27. The antigen binding protein of claim 26, wherein the antigen binding protein binds
human uPAR on human uPAR-expressing cells in the presence of a uPAR ligand.
28. The antigen binding protein of any one of claims 1-23, wherein the antigen binding
protein binds a human uPAR DII-DIII domain or a human uPAR DIII domain.
- 25 29. An isolated antigen binding protein thereof that competes for binding with the antigen
binding protein of any one of claims 1-28.
30. An isolated antigen binding protein thereof that binds the same epitope as the antigen
30 binding protein of any one of claims 1-28.

31. A pharmaceutical composition comprising the antigen binding protein of any one of claims 1-30, and a pharmaceutically acceptable carrier or diluent.
32. An isolated nucleic acid molecule comprising a polynucleotide sequence that encodes the antigen binding protein according to any one of claims 1-30.
33. An expression vector comprising the polynucleotide sequence of claim 32.
34. A host cell comprising the expression vector of claim 33.
35. A method of producing the antigen binding protein of any one of claims 1-30, comprising culturing the host cell of claim 34 under conditions to express the antigen binding protein.
36. The method of claim 35, further comprising isolating the antigen binding protein from the host cell.
37. A use of an antigen binding protein that binds specifically to uPAR, for treating or preventing a disease or disorder associated with uPAR activity or expression, the use comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 31 to a subject in need of such treatment.
38. A method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition of claim 31.
39. A fusion protein comprising:
(i) the antigen binding protein of any one of claims 1-30; and
(ii) a cytokine or variant thereof.
40. The fusion protein of claim 39, wherein the antigen binding protein and the cytokine or variant thereof are linked with an amino acid linker.

41. The fusion protein of claim 39 or 40, wherein the cytokine or variant thereof is selected from the group consisting of IL-2, circularly permuted IL-2 (cpIL-2), IL-15, circularly permuted IL15 (cpIL-15), IL-6, circularly permuted IL-6 (cpIL-6), IL-10, or IL-12.
- 5
42. The fusion protein of claim 39 or 40, wherein the cytokine or variant thereof comprises at least one interleukin-12 (IL-12) subunit.
43. The fusion protein of claim 42, wherein the IL-12 subunit comprises one or both of
- 10 p35 and p40.
44. The fusion protein of claim 43, wherein the p35 comprises one or more amino acid substitutions at positions M12, S27, N28, Q35, F39, S44, C74, M111, V114, M119, M145, F150, L161, and D188 of SEQ ID NO: 81.
- 15
45. The fusion protein of claim 44, wherein the amino acid substitution comprises M12S; S27D; N28R; Q35E; F39D or F39H; S44K; C74K, C74D, C74W, C74Y, or C74Q; M111F or M111W; V114I; M119L; M145L; F150Y; L161D or L161Q; D188N; or a combination thereof.
- 20
46. The fusion protein of claim 44 or 45, wherein the amino acid substitution comprises N28R and Q35E.
47. The fusion protein of any one of claims 44-46, wherein the amino acid substitution
- 25 comprises S27D and D188N.
48. The fusion protein of any one of claims 44-47, wherein the amino acid substitution comprises C74K.
- 30
49. The fusion protein of any one of claims 44-48, wherein the amino acid substitution comprises S27D, C74K, and D188N.

50. The fusion protein of any one of claims 44-49, wherein the p35 comprises an amino acid sequence of SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.
- 5 51. The fusion protein of claim 43, wherein the p40 comprises one or more amino acid substitution at positions C177 and C252 of SEQ ID NO: 84.
52. The fusion protein of claim 51, wherein the C177 amino acid substitution is selected from the group consisting of C177S, C177F, C177M, C177H, C177I, or C177Q.
- 10 53. The fusion protein of claim 51 or 52, wherein the C252 amino acid substitution is C252S.
54. The fusion protein of claim 51 or 52, wherein the p40 comprises an amino acid
15 sequence of SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87.
55. The fusion protein of claim 39 or 40, wherein the cytokine or variant thereof comprises a single-chain IL-12 (scIL-12) comprising a p35 and a p40 subunit attached by an amino acid linker.
- 20 56. The fusion protein of claim 55, wherein the amino acid linker comprises the sequence GGSGGGGSGG (SEQ ID NO: 156).
57. The fusion protein of claim 55 or 56, wherein the scIL-12 comprises an amino acid
25 sequence of SEQ ID NO: 88 or SEQ ID NO: 89.
58. A pharmaceutical composition comprising the fusion protein of any one of claims 39-57, and a pharmaceutically acceptable carrier or diluent.
- 30 59. An isolated nucleic acid molecule comprising a polynucleotide sequence that encodes the fusion protein according to any one of claims 39-57.

60. An expression vector comprising the polynucleotide sequence of claim 59.
61. A host cell comprising the expression vector of claim 60.
- 5 62. A method of producing the fusion protein of any one of claims 39-57, comprising culturing the host cell of claim 61 under conditions to express the fusion protein.
63. The method of claim 62, further comprising isolating the fusion protein from the host
10 cell.
64. A use of fusion protein that binds specifically to uPAR, for treating or preventing a disease or disorder associated with uPAR activity or expression, the use comprising administering a therapeutically effective amount of the pharmaceutical composition of claim
15 58 or the fusion protein of any one of claims 39-57 to a subject in need of such treatment.
65. A method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition of claim 52 or the fusion protein of any one of claims 39-57.
20
66. The method of claim 65, wherein the pharmaceutical composition or fusion protein is administered simultaneously with a cytokine or variant thereof or a second fusion protein, wherein the second fusion protein comprises a different cytokine or variant thereof from the first fusion protein.
- 25 67. The method of claim 65, wherein the pharmaceutical composition or fusion protein is administered sequentially a cytokine or variant thereof or a second fusion protein, wherein the second fusion protein comprises a different cytokine or variant thereof from the first fusion protein.
- 30

68. The method of claim 66 or 67, wherein the cytokine or variant thereof comprises a IL-12 p35 subunit or an IL-12 p40 subunit.

69. The method of any one of claims 65-68, wherein the fusion protein clearance rate in a tumor of the patient is slower than the fusion protein clearance rate in serum of the patient.

70. A method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a fusion protein and a non-fusion IL-12 subunit, wherein:

(a) the fusion protein comprises:

(i) an antigen binding protein that binds specifically to urokinase plasminogen activator receptor (uPAR); and

(ii) a IL-12 p35 subunit or variant thereof, or a IL-12 p40 subunit or variant thereof; and

(b) the non-fusion IL-12 subunit comprising a IL-12 p35 subunit or variant thereof, or a IL-12 p40 subunit or variant thereof,

wherein when the fusion protein comprises a IL-12 p35 subunit or variant thereof, the non-fusion IL-12 subunit comprises a IL-12 p40 subunit or variant thereof, and when the fusion protein comprises a IL-12 p40 subunit or variant thereof, the non-fusion IL-12 subunit comprises a IL-12 p35 subunit or variant thereof.

71. The method of claim 70, wherein the fusion protein and non-fusion IL-12 subunit are administered sequentially.

72. The method of claim 70 or 71, wherein one or both of the fusion protein and non-fusion IL-12 subunit are administered intravenously.

73. The method of any one of claims 70-72, wherein the fusion protein is administered first, followed by administering the non-fusion IL-12 subunit after a sufficient amount of time has elapsed for the fusion protein to accumulate in a tumor of the patient.

74. The method of any one of claims 70-72, wherein the fusion protein is administered first, followed by administering the non-fusion IL-12 subunit after a sufficient amount of time has elapsed for the fusion protein concentration to substantially reduce in serum of the patient.
- 5 75. A method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a first fusion protein and a second fusion protein, wherein:
- (a) the first fusion protein comprises:
- (i) an antigen binding protein that binds specifically to a first epitope on urokinase plasminogen activator receptor (uPAR); and
- 10 (ii) a IL-12 p35 subunit or variant thereof; and
- (b) the second fusion protein comprises:
- (i) an antigen binding protein that binds specifically to a second epitope on uPAR; and
- (ii) a IL-12 p40 subunit or variant thereof.
- 15 76. The method of claim 75, wherein the first epitope and second epitope are the same.
77. The method of claim 75, wherein the first epitope and second epitope are different.
- 20 78. The method of any one of claims 75-77, wherein the first fusion protein and second fusion protein are administered sequentially.
79. The method of any one of claims 75-78, wherein one or both of the first fusion protein and second fusion protein are administered intravenously.
- 25 80. The method of any one of claims 75-79, wherein the first fusion protein is administered first, followed by administering the second fusion protein after a sufficient amount of time has elapsed for the first fusion protein to accumulate in a tumor of the patient.
- 30 81. The method of any one of claims 75-79, wherein the first fusion protein is administered first, followed by administering the second fusion protein after a sufficient

amount of time has elapsed for the first fusion protein concentration to substantially reduce in serum of the patient.

82. The method of any one of claims 75-79, wherein the second fusion protein is
5 administered first, followed by administering the first fusion protein after a sufficient amount of time has elapsed for the second fusion protein to accumulate in a tumor of the patient.

83. The method of any one of claims 75-79, wherein the second fusion protein is
administered first, followed by administering the first fusion protein after a sufficient amount
10 of time has elapsed for the second fusion protein concentration to substantially reduce in serum of the patient.

84. The method of any one of claims 75-83, wherein one or both of the first fusion protein and second fusion protein comprise an antigen binding protein comprising:
15 a) a VH domain comprising an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5); and
b) a VL domain comprising an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), and an LCDR3 amino acid
20 sequence of QQWSSNPPY (SEQ ID NO: 8).

85. The method of any one of claims 75-83, wherein one or both of the first fusion protein and second fusion protein comprise an antigen binding protein comprising:
a) a VH domain comprising an HCDR1 amino acid sequence of GNTFTDYG (SEQ
25 ID NO: 19), or GTTFTDYG (SEQ ID NO: 41), an HCDR2 amino acid sequence of INTNTGEP (SEQ ID NO: 20), an HCDR3 amino acid sequence of AHYSFDY (SEQ ID NO: 21); and
b) a VL domain comprising an LCDR1 amino acid sequence of ENIYSN (SEQ ID NO: 22), an LCDR2 amino acid sequence of AAT (SEQ ID NO: 23), and an LCDR3 amino
30 acid sequence of QHFWGTPWT (SEQ ID NO: 24).

86. The method of any one of claims 75-83, wherein one or both of the first fusion protein and second fusion protein comprise an antigen binding protein comprising:
- a) a VH domain comprising an HCDR1 amino acid sequence of GYTFTSYG (SEQ ID NO: 27), an HCDR2 amino acid sequence of IYPRSGNT (SEQ ID NO: 28), an HCDR3 amino acid sequence of AGKDYGSTYADY (SEQ ID NO: 29); and
 - b) a VL domain comprising an LCDR1 amino acid sequence of SSVSSRY (SEQ ID NO: 30), an LCDR2 amino acid sequence of GTS (SEQ ID NO: 31), and an LCDR3 amino acid sequence of QQYHSDPLT (SEQ ID NO: 32).
87. A combination comprising a first fusion protein and a second fusion protein, wherein:
- (a) the first fusion protein comprises:
 - (i) an antigen binding protein that binds specifically to a first epitope on urokinase plasminogen activator receptor (uPAR); and
 - (ii) a IL-12 p35 subunit or variant thereof; and
 - (b) the second fusion protein comprises:
 - (i) an antigen binding protein that binds specifically to a second epitope on uPAR; and
 - (ii) a IL-12 p40 subunit or variant thereof.
88. The combination of claim 87, wherein the first epitope and second epitope are the same.
89. The combination of claim 87, wherein the first epitope and second epitope are different.
90. A fusion protein comprising:
- (i) an antigen binding protein that binds specifically to urokinase plasminogen activator receptor (uPAR); and
 - (ii) a cytokine or variant thereof.
91. The fusion protein of claim 90, wherein the antigen binding protein and the cytokine or variant thereof are linked with an amino acid linker.

92. The fusion protein of claim 90 or 91, wherein the cytokine or variant thereof is selected from the group consisting of IL-2, circularly permuted IL-2 (cpIL-2), IL-15, circularly permuted IL15 (cpIL-15), IL-6, circularly permuted IL-6 (cpIL-6), IL-10, or IL-12.
- 5
93. The fusion protein of claim 90 or 91, wherein the cytokine or variant thereof comprises at least one interleukin-12 (IL-12) subunit.
94. The fusion protein of claim 93, wherein the IL-12 subunit comprises one or both of
- 10 p35 and p40.
95. The fusion protein of claim 94, wherein the p35 comprises one or more amino acid substitutions at positions M12, S27, N28, Q35, F39, S44, C74, M111, V114, M119, M145, F150, L161, and D188 of SEQ ID NO: 81.
- 15
96. The fusion protein of claim 95, wherein the amino acid substitution comprises M12S; S27D; N28R; Q35E; F39D or F39H; S44K; C74K, C74D, C74W, C74Y, or C74Q; M111F or M111W; V114I; M119L; M145L; F150Y; L161D or L161Q; D188N; or a combination thereof.
- 20
97. The fusion protein of claim 95 or 96, wherein the amino acid substitution comprises N28R and Q35E.
98. The fusion protein of any one of claims 95-97, wherein the amino acid substitution
- 25 comprises S27D and D188N.
99. The fusion protein of any one of claims 95-98, wherein the amino acid substitution comprises C74K.
- 30
100. The fusion protein of any one of claims 95-99, wherein the amino acid substitution comprises S27D, C74K, and D188N.

101. The fusion protein of any one of claims 95-100, wherein the p35 comprises an amino acid sequence of SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.
- 5 102. The fusion protein of claim 94, wherein the p40 comprises one or more amino acid substitution at positions C177 and C252 of SEQ ID NO: 84.
103. The fusion protein of claim 102, wherein the C177 amino acid substitution is selected from the group consisting of C177S, C177F, C177M, C177H, C177I, or C177Q.
- 10 104. The fusion protein of claim 102 or 103, wherein the C252 amino acid substitution is C252S.
105. The fusion protein of any one of claims 102-104, wherein the p40 comprises an amino acid sequence of SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87.
- 15 106. The fusion protein of any one of claims 90-93, wherein the cytokine or variant thereof comprises a single-chain IL-12 (scIL-12) comprising a p35 and a p40 subunit attached by an amino acid linker.
- 20 107. The fusion protein of claim 106, wherein the amino acid linker comprises the sequence GSGGGGGSGG (SEQ ID NO: 156).
108. The fusion protein of claim 106 or 107, wherein the scIL-12 comprises an amino acid sequence of SEQ ID NO: 88 or SEQ ID NO: 89.
- 25 109. The fusion protein of any one of claims 90-108, wherein the antigen binding protein comprises:
- (a) an antibody heavy chain variable (VH) domain comprising:

- i) an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), GFSLTNYG (SEQ ID NO: 11), GNTFTDYG (SEQ ID NO: 19), GTTFTDYG (SEQ ID NO: 41), or GYTFTSYG (SEQ ID NO: 27);
- ii) an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), IWSDGGT (SEQ ID NO: 12), INTNTGEP (SEQ ID NO: 20), or IYPRSGNT (SEQ ID NO: 28); and
- iii) an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5), ARGGRSDLFAY (SEQ ID NO: 13), AHYSFDY (SEQ ID NO: 21), or AGKDYGSTYADY (SEQ ID NO: 29); and
- (b) an antibody light chain variable (VL) domain comprising:
- iv) an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), QSIVHSNGNTY (SEQ ID NO: 14), ENIYSN (SEQ ID NO: 22), SSVSSRY (SEQ ID NO: 30);
- v) an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), KVS (SEQ ID NO: 15), AAT (SEQ ID NO: 23), or GTS (SEQ ID NO: 31); and
- vi) an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8), FQGSHVPYT (SEQ ID NO: 16), QHFWGTPWT (SEQ ID NO: 24), QQYHSDPLT (SEQ ID NO: 32).

110. The fusion protein of claim 109, wherein the VH domain comprises an amino acid sequence of SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 37, SEQ ID NO: 42, or SEQ ID NO: 43, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, or SEQ ID NO: 44.

111. The fusion protein of claim 109, wherein:

a) the VH domain comprises an HCDR1 amino acid sequence of GFNIKDEY (SEQ ID NO: 3), an HCDR2 amino acid sequence of IDPENGDT (SEQ ID NO: 4), an HCDR3 amino acid sequence of TGGNYVGWFPY (SEQ ID NO: 5); and

b) the VL domain comprises an LCDR1 amino acid sequence of SSVSY (SEQ ID NO: 6), an LCDR2 amino acid sequence of DTS (SEQ ID NO: 7), and an LCDR3 amino acid sequence of QQWSSNPPY (SEQ ID NO: 8).

30

112. The fusion protein of claim 109, wherein:

a) the VH domain comprises an HCDR1 amino acid sequence of GFSLTNYG (SEQ ID NO: 11), an HCDR2 amino acid sequence of IWSDGGT (SEQ ID NO: 12), an HCDR3 amino acid sequence of ARGGRSDFAY (SEQ ID NO: 13); and

5 b) the VL domain comprises an LCDR1 amino acid sequence of QSIVHSNGNTY (SEQ ID NO: 14), an LCDR2 amino acid sequence of KVS (SEQ ID NO: 15), and an LCDR3 amino acid sequence of FQGSHVPYT (SEQ ID NO: 16).

113. The fusion protein of claim 109, wherein:

10 a) the VH domain comprises an HCDR1 amino acid sequence of GNTFTDYG (SEQ ID NO: 19), or GTTFTDYG (SEQ ID NO: 41), an HCDR2 amino acid sequence of INTNTGEP (SEQ ID NO: 20), an HCDR3 amino acid sequence of AHYSFDY (SEQ ID NO: 21); and

b) the VL domain comprises an LCDR1 amino acid sequence of ENIYSN (SEQ ID NO: 22), an LCDR2 amino acid sequence of AAT (SEQ ID NO: 23), and an LCDR3 amino acid sequence of QHFWGTPWT (SEQ ID NO: 24).

114. The fusion protein of claim 109, wherein:

20 a) the VH domain comprises an HCDR1 amino acid sequence of GYTFTSYG (SEQ ID NO: 27), an HCDR2 amino acid sequence of IYPRSGNT (SEQ ID NO: 28), an HCDR3 amino acid sequence of AGKDYGSTYADY (SEQ ID NO: 29); and

b) the VL domain comprises an LCDR1 amino acid sequence of SSVSSRY (SEQ ID NO: 30), an LCDR2 amino acid sequence of GTS (SEQ ID NO: 31), and an LCDR3 amino acid sequence of QQYHSDPLT (SEQ ID NO: 32).

25 115. The fusion protein of any one of claims 109-114, wherein:

a) the VH domain comprises an amino acid sequence of SEQ ID NO: 33, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35;

b) the VH domain comprises an amino acid sequence of SEQ ID NO: 33, and the VL domain comprises an amino acid sequence of SEQ ID NO: 36;

30 c) the VH domain comprises an amino acid sequence of SEQ ID NO: 34, and the VL domain comprises an amino acid sequence of SEQ ID NO: 35;

- d) the VH domain comprises an amino acid sequence of SEQ ID NO: 34, and the VL domain comprises an amino acid sequence of SEQ ID NO: 36;
- e) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 38;
- 5 f) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 39;
- g) the VH domain comprises an amino acid sequence of SEQ ID NO: 37, and the VL domain comprises an amino acid sequence of SEQ ID NO: 40;
- h) the VH domain comprises an amino acid sequence of SEQ ID NO: 42, and the VL
10 domain comprises an amino acid sequence of SEQ ID NO: 44; or
- i) the VH domain comprises an amino acid sequence of SEQ ID NO: 43, and the VL domain comprises an amino acid sequence of SEQ ID NO: 44.
116. The fusion protein of claim 115, wherein the VH domain and VL domain are attached
15 with an amino acid linker.
117. The fusion protein of claim 116, wherein the amino acid linker comprises (GGGGS)_n (SEQ ID NO: 148), wherein n is an integer between 1 and 5.
- 20 118. The fusion protein of claim 116, wherein the amino acid linker comprises the amino acid sequence GGGSGGGSGGGGS (SEQ ID NO: 149),
GGSGGGSGGGSGGGSGGGSGGGSGG (SEQ ID NO: 150), GGGSGGGSG (SEQ ID NO: 246).
- 25 119. The fusion protein of any one of claims 90-118, wherein the antigen binding protein comprises a Fab fragment, a F(ab')₂ fragment, an Fd fragment, an Fv fragment, a single chain Fv (scFv), a dAb fragment, a single domain antibody, or a nanobody.
120. The fusion protein of any one of claims 90-119, wherein the antigen binding protein
30 comprises the amino acid sequence of any one of SEQ ID NOs: 56-71.

121. The fusion protein of any one of claims 90-119, wherein the antigen binding protein further comprises the amino acid sequence of any one of SEQ ID NOs: 72-78.
122. The fusion protein of any one of claims 90-119, comprising an antibody heavy chain (HC) amino acid sequence of SEQ ID NO: 45, 46, 49, 53, or 54, and an antibody light chain (LC) amino acid sequence of SEQ ID NO: 47, 48, 50, 51, 52, and 55.
123. The fusion protein of claim 122, wherein:
- a) the antibody HC comprises the amino acid sequence of SEQ ID NO: 45, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 47;
 - b) the antibody HC comprises the amino acid sequence of SEQ ID NO: 45, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 48;
 - c) the antibody HC comprises the amino acid sequence of SEQ ID NO: 46, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 47;
 - d) the antibody HC comprises the amino acid sequence of SEQ ID NO: 46, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 48;
 - e) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 50;
 - f) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 51;
 - g) the antibody HC comprises the amino acid sequence of SEQ ID NO: 49, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 52;
 - h) the antibody HC comprises the amino acid sequence of SEQ ID NO: 53, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 55; or
 - i) the antibody HC comprises the amino acid sequence of SEQ ID NO: 54, and the antibody LC comprises the amino acid sequence of SEQ ID NO: 55.
124. The fusion protein of any one of claims 90-118, comprising an amino acid sequence of SEQ ID NO: 189.

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125. The fusion protein of any one of claims 90-118, comprising an amino acid sequence of SEQ ID NO: 213.
126. The fusion protein of any one of claims 90-118, comprising:
5 a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 178; and
a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159.
127. The fusion protein of any one of claims 90-118, comprising:
a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 193; and
10 a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 161.
128. The fusion protein of any one of claims 90-118, comprising:
a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 179; and
a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159.
15
129. The fusion protein of any one of claims 90-118, comprising:
a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 194; and
a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 162.
- 20 130. The fusion protein of any one of claims 90-118, comprising:
a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 238;
a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 159;
and
a third polypeptide chain comprising an amino acid sequence of SEQ ID NO: 239.
25
131. The fusion protein of any one of claims 90-130, wherein the antigen binding protein binds human uPAR with a KD of less than about 5×10^{-8} M, less than about 1×10^{-8} M, less than about 5×10^{-9} M, less than about 1×10^{-9} M, less than about 5×10^{-10} M, or less than
30 about 1×10^{-10} M.

132. The fusion protein of any one of claims 90-130, wherein the antigen binding protein binds human uPAR in the presence of a uPAR ligand.
133. The fusion protein of any one of claims 90-130, wherein the antigen binding protein
5 binds uPAR ligand-bound human uPAR.
134. The fusion protein of any one of claims 90-130, wherein the antigen binding protein binds human uPAR on human uPAR-expressing cells, thereby producing a fluorescent signal detected with a fluorescently-labelled antibody that binds to the antibody or fragment thereof,
10 with an EC₅₀ value of about 0.1 nM to about 3.0 nM.
135. The fusion protein of any one of claims 90-130, wherein the antigen binding protein binds human uPAR on human uPAR-expressing cells in the presence of a uPAR ligand.
- 15 136. The fusion protein of any one of claims 90-130, wherein the antigen binding protein binds a human uPAR DII-DIII domain or a human uPAR DIII domain.
137. An engineered IL-12 p35 polypeptide, comprising one or more amino acid substitutions at positions S27, N28, Q35, F39, S44, C74, M111, V114, M119, M145, F150,
20 and L161 of SEQ ID NO: 81.
138. The engineered IL-12 p35 polypeptide of claim 137, further comprising one or both of a S27 and D188 amino acid substitution.
- 25 139. The engineered IL-12 p35 polypeptide of claim 137 or 138, wherein the amino acid substitution comprises M12S; S27D; N28R; Q35E; F39D or F39H; S44K; C74K, C74D, C74W, C74Y, or C74Q; M111F or M111W; V114I; M119L; M145L; F150Y; L161D or L161Q; D188N; or a combination thereof.
- 30 140. The engineered IL-12 p35 polypeptide of any one of claims 137-139, wherein the amino acid substitution comprises N28R and Q35E.

141. The engineered IL-12 p35 polypeptide of any one of claims 137-140, wherein the amino acid substitution comprises C74K.
- 5 142. The engineered IL-12 p35 polypeptide of any one of claims 137-141, wherein the amino acid substitution comprises S27D, C74K, and D188N.
143. The engineered IL-12 p35 polypeptide of 137-142, comprising an amino acid sequence of SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.
- 10 144. A fusion protein comprising:
(i) the engineered IL-12 p35 polypeptide of any one of claims 137-143; and
(ii) one or more fusion partners.
- 15 145. The fusion protein of claim 144, wherein the one or more fusion partners comprise a moiety that reduces aggregation of the engineered IL-12 p35 polypeptide.
146. The fusion protein of claim 144, wherein the one or more fusion partners comprise a moiety that increases expression of the engineered IL-12 p35 polypeptide.
- 20 147. The fusion protein of claim 144, wherein the one or more fusion partners comprise a moiety that increases serum half-life of the engineered IL-12 p35 polypeptide.
148. The fusion protein of any one of claims 144-147, wherein the one or more fusion
25 partners comprise a targeting moiety.
149. The fusion protein of any one of claims 144-148, wherein the one or more fusion partners comprise a serum albumin, polyethylene glycol (PEG), or an antigen binding protein.
- 30 150. The fusion protein of claim 149, wherein the antigen binding protein binds specifically to urokinase plasminogen activator receptor (uPAR).

151. An engineered IL-12 p40 polypeptide, comprising one or more amino acid substitutions at positions C177 and C252 of SEQ ID NO: 84.
- 5 152. The engineered IL-12 p40 polypeptide of claim 151, wherein the C177 amino acid substitution is selected from the group consisting of C177S, C177F, C177M, C177H, C177I, or C177Q.
153. The engineered IL-12 p40 polypeptide of claim 151 or 152, wherein the C252 amino
10 acid substitution is C252S.
154. The engineered IL-12 p40 polypeptide of any one of claims 151-153, wherein the p40 comprises an amino acid sequence of SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87.
15
155. A fusion protein comprising:
(i) the engineered IL-12 p40 polypeptide of any one of claims 151-154; and
(ii) one or more fusion partners.
- 20 156. The fusion protein of claim 155, wherein the one or more fusion partners comprise a moiety that reduces aggregation of the engineered IL-12 p40 polypeptide.
157. The fusion protein of claim 155, wherein the one or more fusion partners comprise a moiety that increases expression of the engineered IL-12 p40 polypeptide.
25
158. The fusion protein of claim 155, wherein the one or more fusion partners comprise a moiety that increases serum half-life of the engineered IL-12 p40 polypeptide.
159. The fusion protein of any one of claims 155-158, wherein the one or more fusion
30 partners comprise a targeting moiety.

160. The fusion protein of any one of claims 155-159, wherein the one or more fusion partners comprise a serum albumin, polyethylene glycol (PEG), or an antigen binding protein.
161. The fusion protein of claim 160, wherein the antigen binding protein binds specifically to urokinase plasminogen activator receptor (uPAR).
162. A fusion protein comprising:
(i) a urokinase plasminogen activator receptor (uPAR) binding polypeptide; and
(ii) a cytokine or variant thereof.
163. The fusion protein of claim 162, wherein the uPAR binding polypeptide is linked to the cytokine with an amino acid linker.
164. The fusion protein of claim 162 or 163, wherein the cytokine comprises a functional fragment thereof, functional variant thereof or circularly permuted variant thereof.
165. The fusion protein of any one of claims 162-164, wherein the cytokine is linked via one or more optional amino acid linkers to all or a portion of a receptor for which the cytokine has binding specificity.
166. The fusion protein of any one of claims 162-165, wherein the uPAR binding polypeptide comprises a urokinase plasminogen activator (uPA) polypeptide or variant thereof, capable of specifically binding uPAR.
167. The fusion protein of claim 166, wherein the uPA variant is an amino acid terminal fragment (ATF) of uPA.
168. The fusion protein of claim 167, wherein the ATF has the amino acid sequence as set forth in SEQ ID NO: 91, or an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 91.

169. The fusion protein of claim 167 or 168, wherein the ATF of uPA comprises one or more amino acid substitutions or deletions.
170. The fusion protein of claim 166, wherein the uPA has the amino acid sequence set forth in SEQ ID NO: 90, or an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 90.
171. The fusion protein of any one of claims 162-170, wherein the cytokine is circularly permuted IL-2 and the receptor is IL-2R α .
172. The fusion protein of any one of claims 162-170, wherein the cytokine is circularly permuted IL-15 and the receptor is IL-15R α .
173. The fusion protein of any one of claims 162-170, wherein the cytokine is circularly permuted IL-1 and the receptor is IL-1RI, IL-1RII.
174. The fusion protein of any one of claims 162-165, wherein the uPAR binding polypeptide is an anti-uPAR antigen binding protein.
175. The fusion protein of any one of claims 162-165, wherein the uPAR binding polypeptide is an scFv fragment of an anti-uPAR antibody.
176. The fusion protein of any one of claims 162-165, selected from the group consisting of: SEQ ID NOs: 98, 99, 100, 106, 107, 111, and 113.
177. The fusion protein of any one of claims 162-165, selected from the group consisting of SEQ ID NO: 98, 99, 111, and 112.
178. The fusion protein of any one of claims 162-177, wherein the uPAR binding agent, cytokine and receptor are of human origin.

179. A method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a fusion protein of any one of claims 162-178.
- 5 180. The method of claim 179, wherein the cancer is selected from the group consisting of: melanoma, carcinoma, blastoma and lymphoma.
181. A method of treating an inflammatory disease in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a fusion protein of any one
10 of claims 162-178.
182. The method of claim 181, wherein the inflammatory disease is selected from the group consisting of IBD and RA.
- 15 183. A pharmaceutical composition comprising at least one fusion protein of any one of claims 162-178 and optionally a pharmaceutically acceptable excipient.
184. A fusion protein comprising:
a urokinase plasminogen activator receptor (uPAR) binding polypeptide linked via one
20 or more optional amino acid linkers to:
(i) a cytokine or variant thereof; or
(ii) a checkpoint molecule modulator.
185. The fusion protein of claim 184, wherein the cytokine comprises a functional fragment
25 thereof, functional variant thereof or circularly permuted variant thereof.
186. The fusion protein of claims 184 or 185, wherein the cytokine is linked via one or more optional amino acid linkers to all or a portion of a receptor for which the cytokine has binding specificity.
30

187. The fusion protein of any one of claims 184-186, wherein the uPAR binding polypeptide comprises a urokinase plasminogen activator (uPA) polypeptide or variant thereof, capable of specifically binding uPAR.
- 5 188. The fusion protein of claim 187, wherein the uPA variant is an amino acid terminal fragment (ATF) of uPA.
189. The fusion protein of claim 187 or 188, wherein the ATF has the amino acid sequence as set forth in SEQ ID NO: 91, or an amino acid sequence with at least 80% identity to the
10 amino acid sequence as set forth in SEQ ID NO: 91.
190. The fusion protein of claim 188 or 189, wherein the ATF of uPA comprises one or more amino acid substitutions or deletions.
- 15 191. The fusion protein of any one of claims 184-190, wherein the cytokine or checkpoint molecule modulator are selected from the group consisting of: IL-2, circularly permuted IL-2 (cpIL-2), IL-15, circularly permuted IL15 (cpIL-15), IL-6, circularly permuted IL-6 (cpIL-6), IL-10, IL-12, IL-15, GM-CSF, IFN γ , IFN α , 4-1BBB (CD131) or ligand thereof, OX40 or ligand thereof, PD-1, PD-L1, anti-PD-1 antibody, CTLA-4, anti-CTLA4 antibodies, GITR or
20 ligand thereof, ICOS or ligand thereof, CD27, CD70, CD40 or ligand thereof.
192. A method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a fusion protein of any one of claims 184-191.
25
193. The method of claim 192, wherein the cancer is selected from the group consisting of: melanoma and lymphoma.
194. A method of treating an inflammatory disease in a patient in need thereof comprising
30 administering to the patient a therapeutically effective amount of a fusion protein of any one of claims 184-191.

195. The method of claim 194, wherein the inflammatory disease is selected from the group consisting of IBD and RA.
- 5 196. A pharmaceutical composition comprising at least one fusion protein of any one of claims 184-191 and optionally a pharmaceutically acceptable excipient.
197. A method of immunotherapy for treating cancer in a patient comprising administering a fusion protein of claims 162 or 184 to a patient in need thereof.
- 10 198. The method of claim 197, further comprising administering a targeted cancer therapy to the patient.
199. The method of claim 198, further comprising administering a conventional cytotoxic
15 therapy to the patient selected from chemotherapy, radiation therapy or both.
200. The method of any one of claims 197-199, further comprising administering an immunotherapy selected from a cancer vaccine, a checkpoint inhibitor or a monoclonal antibody.
- 20 201. The fusion protein of claims 162 or 184, wherein the uPAR binding polypeptide comprises M25 or an amino acid sequence homologous thereto having at least 80% identity at the amino acid level.
- 25 202. The fusion protein of claims of claims 162 or 184, wherein the uPAR binding polypeptide comprises P25 or an amino acid sequence homologous thereto having at least 80% identity at the amino acid level.
- 30