

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 acid sequence of SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 37, SEQ ID NO: 42, or SEQ
25 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, optionally 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;

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;

5 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;

10 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 domain comprises an amino acid sequence of SEQ ID NO: 44; or

15 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.

3. The antigen binding protein of claim 1, wherein:

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 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);

25 b) 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 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);

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

d) 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 the VL domain comprises an
10 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).

4. The antigen binding protein of claim 1, wherein the VH domain and VL domain are
15 attached with an amino acid linker.

5. The antigen binding protein of any one of claims 1-4, 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.
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~~6.~~ The antigen binding protein of any one of claims 1-4, comprising the amino acid sequence of any one of SEQ ID NOs: 56-71, optionally further comprising the amino acid sequence of any one of SEQ ID NOs: 72-78.

25 7. The antigen binding protein of any one of claims 1-6, 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, optionally 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;

5 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;

10 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

15 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.

8. The antigen binding protein of any one of claims 1-4, wherein:

the antigen binding protein comprises an amino acid sequence of SEQ ID NO: 189;

20 the antigen binding protein comprises an amino acid sequence of SEQ ID NO: 213;

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;

25 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;

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;

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; or

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.

9. The antigen binding protein of any one of claims 1-8, 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 about 1×10^{-10} M;

the antigen binding protein binds human uPAR in the presence of a uPAR ligand;

the antigen binding protein thereof binds uPAR ligand-bound human uPAR;

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, optionally wherein the antigen binding protein binds human uPAR on human uPAR-expressing cells in the presence of a uPAR ligand; and/or

the antigen binding protein binds a human uPAR DII-DIII domain or a human uPAR DIII domain.

10. A pharmaceutical composition comprising the antigen binding protein of any one of claims 1-9, and a pharmaceutically acceptable carrier or diluent.

11. An isolated nucleic acid molecule comprising a polynucleotide sequence that encodes the antigen binding protein according to any one of claims 1-9.

12. An expression vector comprising the polynucleotide sequence of claim 11.

13. A host cell comprising the expression vector of claim 12.

14. A method of producing the antigen binding protein of any one of claims 1-9, comprising culturing a host cell comprising an expression vector comprising a polynucleotide
5 sequence that encodes the antigen binding protein- under conditions to express the antigen binding protein, optionally wherein the method further comprises isolating the antigen binding protein from the host cell.

15. A method of treating cancer in a patient in need thereof comprising administering
10 to the patient a therapeutically effective amount of the pharmaceutical composition of claim 10.

16. A fusion protein comprising:

(i) the antigen binding protein of any one of claims 1-9; and

(ii) a cytokine or variant thereof,

15 optionally wherein the antigen binding protein and the cytokine or variant thereof are linked with an amino acid linker.

17. The fusion protein of claim 16, wherein:

the cytokine or variant thereof is selected from the group consisting of IL-2, circularly
20 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;

the cytokine or variant thereof comprises at least one interleukin-12 (IL-12) subunit, optionally wherein the IL-12 subunit comprises one or both of p35 and p40; and/or

the cytokine or variant thereof comprises a single-chain IL-12 (scIL-12) comprising a
25 p35 and a p40 subunit attached by an amino acid linker, optionally wherein the amino acid linker comprises the sequence GGS GGG GSGG (SEQ ID NO: 156), or the scIL-12 comprises an amino acid sequence of SEQ ID NO: 88 or SEQ ID NO: 89.

18. The fusion protein of claim 17, 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, optionally wherein:

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

the amino acid substitution comprises N28R and Q35E;

the amino acid substitution comprises S27D and D188N;

the amino acid substitution comprises C74K;

10 the amino acid substitution comprises S27D, C74K, and D188N; or

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

19. The fusion protein of claim 17, wherein the p40 comprises one or more amino acid substitution at positions C177 and C252 of SEQ ID NO: 84, optionally wherein:

the C177 amino acid substitution is selected from the group consisting of C177S, C177F, C177M, C177H, C177I, or C177Q;

the C252 amino acid substitution is C252S; and/or

20 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.

20. A method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of the fusion protein of any one of claims 16-19, optionally wherein:

25 the fusion protein is administered simultaneously or sequentially 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; and/or

the fusion protein clearance rate in a tumor of the patient is slower than the fusion protein clearance rate in serum of the patient.

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21. 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.

22. The method of claim 21, wherein:

the fusion protein and non-fusion IL-12 subunit are administered sequentially;

one or both of the fusion protein and non-fusion IL-12 subunit are administered intravenously;

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; and/or

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.

23. 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

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

(b) the second fusion protein comprises:

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

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

24. The method of claim 23, wherein:

10 the first epitope and second epitope are the same or different;

the first fusion protein and second fusion protein are administered sequentially;

one or both of the first fusion protein and second fusion protein are administered intravenously;

15 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;

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;

20 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; or

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.

25 25. The method of claim 23 or 24, wherein 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 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);

5 b) 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 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
10 QHFWGTPWT (SEQ ID NO: 24); or

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

26. A combination comprising a first fusion protein and a second fusion protein, wherein:

20 (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:

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

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

 optionally wherein the first epitope and second epitope are the same or different.

30 27. 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,

optionally wherein the antigen binding protein and the cytokine or variant thereof are
5 linked with an amino acid linker.

28. The fusion protein of claim 27, 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
10 IL-6 (cpIL-6), IL-10, or IL-12;

the cytokine or variant thereof comprises at least one interleukin-12 (IL-12) subunit,
optionally wherein the IL-12 subunit comprises one or both of p35 and p40; and/or

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, optionally wherein the amino acid
15 linker comprises the sequence GSGGGGSGG (SEQ ID NO: 156), or the scIL-12 comprises
an amino acid sequence of SEQ ID NO: 88 or SEQ ID NO: 89.

29. The fusion protein of claim 28, wherein the p35 comprises one or more amino acid
substitutions at positions M12, S27, N28, Q35, F39, S44, C74, M111, V114, M119, M145,
20 F150, L161, and D188 of SEQ ID NO: 81, optionally 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;

the amino acid substitution comprises N28R and Q35E;

25 the amino acid substitution comprises S27D and D188N;

the amino acid substitute comprises C74K;

the amino acid substitution comprises S27D, C74K, and D188N; pr

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

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30. The fusion protein of claim 28, wherein the p40 comprises one or more amino acid substitution at positions C177 and C252 of SEQ ID NO: 84, optionally wherein:

the C177 amino acid substitution is selected from the group consisting of C177S, C177F, C177M, C177H, C177I, or C177Q;

5 the C252 amino acid substitution is C252S; and/or

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.

31. The fusion protein of any one of claims 27-30, wherein the antigen binding protein
10 comprises an antibody heavy chain variable (VH) domain and an antibody light chain variable (VL) domain, 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 the VL domain comprises an LCDR1
15 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);

b) 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 the VL domain comprises an
20 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);

c) 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
25 (SEQ ID NO: 20), an HCDR3 amino acid sequence of AHYSFDY (SEQ ID NO: 21); and 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); or

d) the VH domain comprises an HCDR1 amino acid sequence of GYTFTSYG (SEQ ID
30 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 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).

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32. The fusion protein of claim 31, 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;

10 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;

15 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;

20 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.

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33. 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, L161, and D188 of SEQ ID NO: 81.

34. The engineered IL-12 p35 polypeptide of claim 33, 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, optionally wherein:

5 the amino acid substitution comprises N28R and Q35E;

 the amino acid substitution comprises C74K;

 the amino acid substitution comprises S27D, C74K, and D188N; and/or

 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.

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35. An engineered IL-12 p40 polypeptide, comprising one or more amino acid substitutions at positions C177 and C252 of SEQ ID NO: 84.

36. The engineered IL-12 p40 polypeptide of claim 35, wherein the C177 amino acid substitution is selected from the group consisting of C177S, C177F, C177M, C177H, C177I, or C177Q;

 the C252 amino acid substitution is C252S; and/or

 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.

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