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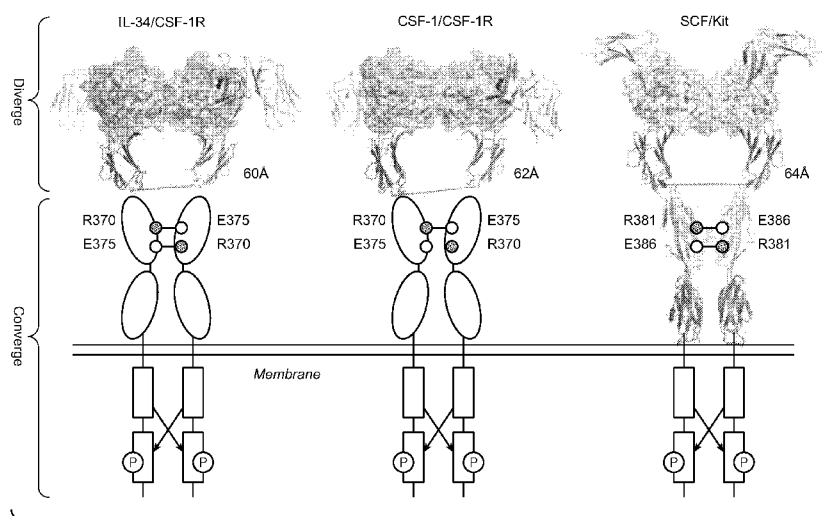
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(54) Title: COMPOSITIONS AND METHODS FOR USING CSF1R INHIBITORS

**FIG. 6**

(57) **Abstract**: The invention provides CSF1-R pathway inhibitors, including anti-IL-34 antibodies, bispecific anti-IL-34/CSF-1 antibodies and anti-CSF-1R antibodies and methods of using the same for treating myeloid pathogenic immunological diseases.



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## COMPOSITIONS AND METHODS FOR USING CSF1R INHIBITORS

### FIELD OF THE INVENTION

The present invention relates to compositions and methods for using CSF1-R pathway inhibitors, including anti-IL-34 antibodies, bispecific IL-34/CSF1 antibodies and CSF1R antibodies.

### BACKGROUND

Interleukin-34, also known as C16orf77 or UNQ20374 (Clark et al., *Genome Res* **13**: 2265-2270 (2003)), was recently identified as a second and high-affinity ligand for CSF-1R in a human monocyte proliferation screening (Lin et al., *Science* **320**: 807-811 (2008)). This discovery has long been foreshadowed by the more severe phenotype in CSF-1R null mice, than CSF-1-deficient CSF-1<sup>op</sup>/CSF-1<sup>op</sup> mice (Dai et al., *Blood* **99**: 111-120 (2002)). Like CSF-1 (also known as M-CSF), the better-characterized ligand for CSF-1R, IL-34 stimulates phosphorylation of ERK1/2 in human monocytes and promotes the formation of the granulocyte-macrophage progenitor (CFU-GM) and megakaryocyte progenitor (CFU-M) in human bone marrow cultures (Lin et al., *Science* **320**: 807-811 (2008)). Mediated by the common receptor CSF-1R, the transcript of the proto-oncogene c-fms, IL-34 and CSF-1 serve as the key regulators of the differentiation, proliferation, and survival of the mononuclear phagocyte lineage cell such as monocytes, macrophages and osteoclasts (Droin et al., *Journal of leukocyte biology* **87**: 745-747 (2010)).

The function of IL-34 bears strong resemblance to that of CSF-1, but with several notable differences. Both cytokines support cell growth and survival in cell cultures studies equivalently (Chihara et al., *Cell death and differentiation* **17**: 1917-1927 (2010); Wei et al., *Journal of leukocyte biology* **88**: 495-505 (2010)). The IL-34 gene, when expressed under the control of the CSF-1 promoter, could rescue the phenotype of CSF-1-nullizygous CSF-1<sup>op</sup>/CSF-1<sup>op</sup> mice (Wei et al., *Journal of leukocyte biology* **88**: 495-505 (2010)). IL-34 can also substitute for CSF-1 to support RANKL-induced osteoclastogenesis (Baud'huin et al., *The Journal of pathology* **221**: 77-86 (2010)). However, the two factors appear different in their ability to induce the production of chemokines such as MCP-1 and eotaxin-2 in primary macrophages, the morphological change in TF-1-fms cells and the migration of J774A.1 cells (Chihara et al., *Cell death and differentiation* **17**: 1917-1927 (2010)). IL-34 has been shown

to induce a stronger, but transient tyrosine phosphorylation of CSF-1R and downstream effectors, and rapidly downregulates CSF-1R expression (Chihara et al., *Cell death and differentiation* **17**: 1917-1927 (2010)). Moreover, IL-34 and CSF-1 exhibit differential spatiotemporal patterns of expression in both embryonic and adult tissues, which leads to the complementary activation of the CSF-1R (Wei et al., *Journal of leukocyte biology* **88**: 495-505 (2010)). Most strikingly, IL-34 but not CSF-1 messenger RNA is detected together with CSF-1R in embryonic brain which could explain why microglia develop in CSF-1 deficient but not CSF-1R deficient mice (Ginhoux et al., *Science* **330**: 841-845 (2010); Mizuno et al., *The American journal of pathology* **179**: 2016-2027 (2011)). Thus, while IL-34 and CSF-1 resemble each other, they are not necessarily identical in their developmental roles, biological activity, and signal activation kinetics or strength.

Despite a lack of appreciable sequence similarity with other proteins, IL-34 was proposed by fold recognition methods to be a short-chain helical cytokine belonging to the same family as CSF-1, SCF, and Flt3L (Garceau et al., *Journal of leukocyte biology* **87**: 753-764 (2010)).

These latter three dimeric hematopoietic cytokines are unique among helical cytokines in that they have membrane-bound forms (Bazan, *Cell* **65**: 9-10 (1991a); Hannum et al., *Nature* **368**: 643-648 (1994)); IL-34 differs importantly in that it lacks a hydrophobic transmembrane segment. In addition, CSF-1, SCF and Flt3L cytokine dimers bind to the PDGFR subfamily (type III/V) of the receptor tyrosine kinase (RTK) family (Rosnet et al., *Critical reviews in oncogenesis* **4**: 595-613 (1993)) instead of hematopoietic cytokine receptors (Bazan, *Immunology today* **11**: 350-354 (1990)). The CSF-1, SCF and Flt3L cytokine dimers functionally mimic the PDGF and VEGF cystine knot growth factor dimers that are the activating ligands of the RTK family (Savvides et al., *Nature structural biology* **7**: 486-491 (2000); Wiesmann et al., *Nature structural biology* **7**: 440-442 (2000)). All members of this RTK family share a similar overall architecture comprised of multiple Ig-like domains in their extracellular regions, a single transmembrane segment, and a cytoplasmic tyrosine kinase domain with a large insertion. Upon stimulation, CSF-1R dimerizes and autophosphorylates certain tyrosine residues in its intracellular domain, which serve as docking sites for SH2-containing effector proteins, which contribute to macrophage differentiation (Pixley et al., *Trends in cell biology* **14**: 628-638 (2004)).

The structure of dimeric CSF-1 in complex with CSF-1R reveals a non-symmetrical 2:1 complex, in which one CSF-1 protomer approaches its receptor at the cleft between D2 and D3, while the second CSF-1 protomer remains unoccupied (Chen et al., *Proceedings of the*



*National Academy of Sciences of the United States of America* **105**: 18267-18272 (2008)).

Yet, the molecular basis whereby CSF-1R is also able to recognize IL-34, a distantly-related ligand with scant sequence identity, has remained elusive until described herein. IL-34 can function independently, but does not synergize with CSF-1 (Lin et al., *Science* **320**: 807-811 (2008)). Indeed, CSF-1 competes with IL-34 for binding to CSF-1R (Wei et al., *Journal of leukocyte biology* **88**: 495-505 (2010)), suggesting a common ligand-binding site on CSF-1R. In contrast however, a recent comparative sequence study between CSF-1R and its two ligands suggested the CD loop of IL-34, and the junction between D3 and D4 of CSF-1R share strong sequence conservation correlation coefficients during evolution, and therefore may represent a unique binding mode that is distinct from the binding mode employed by the CSF-1/CSF-1R complex (Garceau et al., *Journal of leukocyte biology* **87**: 753-764 (2010)). Interestingly, an anti-CSF-1R antibody MAb 12-2D6 was reported to block the binding of both IL-34 and CSF-1 to CSF-1R, yet another antibody MAb 2-4A5 blocked only CSF-1/CSF-1R binding (Chihara et al., *Cell death and differentiation* **17**: 1917-1927 (2010)). These results strongly suggest that IL-34 and CSF-1 have overlapping, but not identical binding sites on the surface of CSF-1R.

All references cited herein, including patent applications and publications, are hereby incorporated by reference in their entirety.

## SUMMARY

The invention provides anti-IL-34 antibodies, bispecific antibodies that bind to IL-34 and CSF-1, and anti-CSF-1R antibodies, and methods of using the same. In one embodiment, the antibodies of this invention have reduced antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) activity. In one specific embodiment, the antibodies of this invention have reduced ADCC activity by comprising at least an Fc region substitution at one or more of the following residues 238, 265, 269, 270, 297, 327 and 329 (EU numbering). In one specific embodiment, the Fc region substitution to reduce ADCC activity is at residue 297. In another embodiment, the Fc region substitution to reduce ADCC activity is N297G or N297A. In another embodiment, the Fc substitution to reduce ADCC is D265A. In yet another embodiment, the Fc substitutions to reduce ADCC activity are the substitution of residues 265 and 297 to alanine. In one embodiment, the bispecific anti-IL-34/anti-CSF-1 antibody is a knob-into-hole bispecific antibody.

Provided herein are isolated antibodies that bind to human IL-34, which bind to an epitope comprising at least one of amino acid residues Glu103, Leu109, Gln106, Asn150, Leu127, Asn128, Ser184, Leu186, Asn187, Lys44, Glu121, Asp107, Glu111, Ser104, Gln120, Trp116, and Asn61 of a human IL-34, where the position of the amino acid residues is based on the position in SEQ ID NO:1, and which inhibit the binding between human IL-34 and human CSF-1R.

Provided herein are isolated antibodies that binds to human IL-34, which bind to an epitope comprising at least one of amino acid residues from Glu103 to Asn150 of a human IL-34, where the position of the amino acid residues is based on SEQ ID NO:1, and which inhibit the binding between human IL-34 and human CSF-1R.

In some embodiments, the antibody binds to an epitope comprising at least one of amino acid residues Glu103, Leu109, Gln106, and Asn150 of the human IL-34, where the position of the amino acid residues is based on the position in SEQ ID NO:1. In some embodiments, the epitope further comprises at least one of amino acid residues Ser100, Glu123, Trp116, Thr124, Leu127, Asn128, Gln131, and Thr134 of the human IL-34, where the position of the amino acid residues is based on the position in SEQ ID NO:1. In some embodiments, the antibody binds to amino acids within positions 100-108, 116-134, 109 and 150 of the human IL-34, where the position of the amino acid residues is based on the position in SEQ ID NO:1.

In some embodiments, the antibody binds to an epitope comprising at least one of amino acid residues Asn128, Ser184, Leu186, Asn187, Lys44, and Glu121 of the human IL-34, where the position of the amino acid residues is based on the position in SEQ ID NO:1. In some embodiments, the epitope further comprises at least one of amino acid residues Phe40, Asp43, Leu125, Gln189, Thr36, and Val185 of the human IL-34, where the position of the amino acid residues is based on the position in SEQ ID NO:1. In some embodiments, the antibody binds to amino acids within positions 36-44, 121-128, and 184-187 of the human IL-34, where the position of the amino acid residues is based on the position in SEQ ID NO:1.

In some embodiments, the antibody binds to an epitope comprising at least one of amino acid residues from Glu103-Leu127 of the human IL-34, where the position of the amino acid residues is based on the position in SEQ ID NO:1. In some embodiments, the antibody binds to an epitope comprising at least one of amino acid residues Asp107, Glu111, Ser104, Gln120, Glu103, Leu109, Trp116, and Asn61 of the human IL-34, where the position of the amino acid residues is based on the position in SEQ ID NO:1. In some embodiments, the

epitope further comprises at least one of amino acid residues Pro152, Val108, Leu110, Gln106, Glu123, Leu127, Lys117, Ile60 and Lys55 of the human IL-34, where the position of the amino acid residues is based on the position in SEQ ID NO:1. In some embodiments, the antibody binds to amino acids within positions 55-61, 100-108, 109, 111-127 and 152 of the human IL-34, where the position of the amino acid residues is based on the position in SEQ ID NO:1.

In some embodiments, the antibody comprises a heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:3 and/or a light chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ

ID NO:4. In some embodiments, the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:3 and/or a light chain variable region sequence of the amino acid sequence of SEQ ID NO:4. In some embodiments, the antibody comprises (a) a HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); (b) a HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39); and (c) a HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ

ID NO: 52). In some embodiments, the antibody comprises (a) a HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59), (b) a HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52); and (c) a HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33). In some embodiments, the

antibody comprises (a) a HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (b) a HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) a HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39).

In some embodiments, the antibody comprises (a) a HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33) or GINQGSKRGAMDY (SEQ ID NO: 32); (b) a HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39) or QQSYTTPPT (SEQ ID NO: 43) or QQYTALPYT (SEQ ID NO: 49) or QQYSDLPYT (SEQ ID NO: 45) or QQYSDVPYT (SEQ ID NO: 47) or QQSRTARPT (SEQ ID NO: 41); and (c) a HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52)

or RISPYSGYTNYADSVKG (SEQ ID NO: 51). In some embodiments, the antibody comprises (a) a HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59); (b) a HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52) or RISPYSGYTNYADSVKG (SEQ ID NO: 51); and (c) a HVR-H3 comprising an amino

acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33) or GINQGSKRGAMDY (SEQ ID NO: 32). In some embodiments, the antibody comprises (a) a HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (b) a HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) a HVR-L3 comprising an amino acid

sequence QQSFYFPNT (SEQ ID NO: 39) or QQSYTTPPT (SEQ ID NO: 43) or QQYTALPYT (SEQ ID NO: 49) or QQYSDLPYT (SEQ ID NO: 45) or QQYSDVPYT (SEQ ID NO: 47) or QQSRTARPT (SEQ ID NO: 41) or QQSFYFPN (SEQ ID NO: 38) or QQSYTTPP (SEQ ID NO: 42) or QQYTALPY (SEQ ID NO: 48) or QQYSDLPY (SEQ ID NO: 44) or QQYSDVPY (SEQ ID NO: 46) or QQSRTARP (SEQ ID NO: 40).

In some embodiments, the antibody comprises (a) a HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); (b) a HVR-L3 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45); and (c) a HVR-H2 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ ID NO: 51). In some embodiments, the antibody comprises (a) a HVR-H1 comprising an amino acid sequence of STWIH (SEQ ID NO: 59);

(b) a HVR-H2 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ ID NO: 51); and (c) a HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33). In some embodiments, the antibody comprises (a) a HVR-L1 comprising an amino acid sequence of RASQDVSTAVA (SEQ ID NO: 50); (b) a HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) a HVR-L3 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45).

In some embodiments, the antibody comprises a heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:5 and/or a light chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:6. In some embodiments, the antibody comprises a heavy chain variable region

sequence of the amino acid sequence of SEQ ID NO:5 and/or a light chain variable region sequence of the amino acid sequence of SEQ ID NO:6. In some embodiments, the antibody comprises a heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:7 and/or a light chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:8. In some embodiments,

the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:7 and/or a light chain variable region sequence of the amino acid sequence of SEQ ID NO:8. In some embodiments, the antibody comprises a heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:9

and/or a light chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:10. In some embodiments, the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:9 and/or a light chain variable region sequence of the amino acid sequence of SEQ ID NO:10. In some

embodiments, the antibody comprises a heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11 and/or a light chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID

NO:12. In some embodiments, the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:11 and/or a light chain variable region

sequence of the amino acid sequence of SEQ ID NO:12. In some embodiments, the antibody comprises a heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:13 and/or a light chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:14. In some

embodiments, the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:13 and/or a light chain variable region sequence of the amino acid sequence of SEQ ID NO:14.

In some embodiments, the antibody comprises (a) a HVR-H3 comprising an amino acid sequence SRGAYRFAY (SEQ ID NO: 56); (b) a HVR-L3 comprising an amino acid sequence QQSYTTPPT (SEQ ID NO: 43); and (c) a HVR-H2 comprising an amino acid

sequence SITPASGDDYADSVKG (SEQ ID NO: 54). In some embodiments, the antibody comprises (a) a HVR-H1 comprising an amino acid sequence SNYIH (SEQ ID NO: 55), (b) a HVR-H2 comprising an amino acid sequence SITPASGDDYADSVKG (SEQ ID NO: 54); and (c) a HVR-H3 comprising an amino acid sequence SRGAYRFAY (SEQ ID NO: 56). In

some embodiments, the antibody comprises (a) a HVR-L1 comprising an amino acid

sequence RASQDVSTAVA (SEQ ID NO: 50); (b) a HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) a HVR-L3 comprising an amino acid sequence QQSYTTPPT (SEQ ID NO: 43). In some embodiments, the antibody comprises a

heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:15 and/or a light chain variable region sequence of at least 90%

sequence identity to the amino acid sequence of SEQ ID NO:16. In some embodiments, the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:15 and/or a light chain variable region sequence of the amino acid sequence of

SEQ ID NO:16. In some embodiments, the antibody does not inhibit the binding between human CSF-1 and human CSF-1R.

In some embodiments, the anti-IL-34 antibody described herein binds to a dimer of the IL-34.

In some embodiments, the anti-IL-34 antibody described herein binds to an epitope that spans

5 over both protomers of the IL-34 dimer. In some embodiments, the anti-IL-34 antibody described herein neutralizes IL-34 activity. In some embodiments, the anti-IL-34 antibody binds to human IL-34, inhibit the binding between human IL-34 and human CSF-1R, and/or neutralize IL-34 activity.

In some embodiments, the anti-IL-34 antibody described herein is a monoclonal antibody. In  
10 some embodiments, the anti-IL-34 antibody described herein a human, humanized or chimeric antibody. In some embodiments, the antibody is a bispecific antibody. In some embodiments, the bispecific antibody comprises a second binding specificity to human CSF-1.

Provided herein are bispecific antibodies comprising a first binding specificity to human IL-  
15 34 and a second binding specificity to human CSF-1 and their use in treating myeloid pathogenic immunological diseases and cancers. In some embodiments, the antibody inhibits binding of human IL-34 to human CSF-1R and inhibits binding of human CSF-1 to human CSF-1R. In another embodiment, provided herein are two polypeptides comprising binding specificity to human IL-34 and the binding specificity to human CSF-1, respectively, each has  
20 a heteromultimerization domain that is capable is heterodimerizing with each other.

In some embodiments, the antibody described above is an antibody fragment that binds human IL-34. In some embodiments, the fragment is a Fab, Fab', Fab'-SH, F(ab')<sub>2</sub>, Fv or scFv fragment.

In some embodiments, the antibody described herein is a one-armed antibody. In some  
25 embodiments, the antibody described herein is a linear antibody. In some embodiments, the antibody described herein is a full length IgG1 or an IgG4 antibody.

Further provided herein are isolated antibodies that bind human CSF-1R, which bind to an epitope comprising at least one of amino acid residues Arg144, Gln248, Gln249, Ser250, Phe252, and Asn254 of human CSF-1R, where the position of amino acid residue is based on  
30 the position in SEQ ID NO:2, and which inhibit the binding between human IL-34 and human CSF-1R.

In some embodiments, the antibody binds to an epitope comprising amino acid residue Arg144 of CSF-1R, where the position of amino acid residue is based on the position in SEQ

ID NO:2. In some embodiments, the antibody binds to an epitope comprising at least one of amino acid residues Arg144, Arg142, Arg146, and Arg150 of human CSF-1R, where the position of amino acid residues is based on the position in SEQ ID NO:2. In some embodiments, the epitope further comprises at least one of amino acid residues Ser172 and Arg192 of human CSF-1R, where the position of amino acid residues is based on the position in SEQ ID NO:2. In some embodiments, the epitope further comprises at least one of amino acid residues Arg146, Met149, Arg150, Phe169, Ile170, and Gln173 of human CSF-1R, where the position of amino acid residues is based on the position in SEQ ID NO:2. In some embodiments, the antibody binds to amino acids within positions 142-150 and 169-173, where the position of amino acid residues is based on the position in SEQ ID NO:2.

In some embodiments, the antibody binds to an epitope comprising at least one of amino acid residues Arg144, Gln248, Gln249, Ser250, Phe252, and Asn254 of human CSF-1R, where the position of amino acid residue is based on the position in SEQ ID NO:2. In some embodiments, the antibody binds to an epitope comprising at least one of amino acid residues Tyr257, Gln248, Gln249, Ser250, Phe252, and Asn254 of human CSF-1R, where the position of amino acid residues is based on the position in SEQ ID NO:2. In some embodiments, the epitope further comprises at least one of amino acid residues Pro247, Gln258, and Lys259, , where the position of amino acid residues is based on the position in SEQ ID NO:2. In some embodiments, the epitope further comprises at least one of amino acid residues Val231, Asp251, and Tyr257 of human CSF-1R, where the position of amino acid residue is based on the position in SEQ ID NO:2. In some embodiments, the antibody binds to amino acid residues within positions 231, 248-252, and 254, where the position of amino acid residues is based on the position in SEQ ID NO:2.

Further provided herein are isolated nucleic acids encoding any of the antibodies described herein. Also provided herein are vectors comprising the nucleic acid of any of the nucleic acids provided herein. Also provided herein are host cells comprising the nucleic acid provided herein.

Further provided herein are methods of producing an antibody, comprising culturing any of the host cells provided herein, so that the antibody is produced. In some embodiments, the method further comprises recovering the antibody produced by the host cell.

Also provided herein are pharmaceutical compositions comprising any of the antibodies provided herein and a pharmaceutically acceptable carrier.

Provided herein are antibodies described herein for use as a medicament. Provided herein are the antibodies described herein for use in treating a myeloid pathogenic immunological disease. Provided herein are the antibodies described herein for use in inhibiting binding between human IL-34 and human CSF-1R.

- 5 Provided herein is also use of any of the antibodies described herein in the manufacture of a medicament. In some embodiments, the medicament is for treating a myeloid pathogenic immunological disease. In some embodiments, the medicament is for inhibiting binding between human IL-34 and human CSF-1R.

- 10 Provided herein are methods of treating an individual having an inflammatory disease and/or an autoimmune disease with a myeloid pathogenic component ("myeloid pathogenic immunological disease") comprising administering to the individual an effective amount of any one of the antibodies provided herein. Also provided herein are methods of treating an individual having an inflammatory disease and/or an autoimmune disease comprising administering to the individual an effective amount of any one of the antibodies or
- 15 combination therapies provided herein. In some embodiments, the antibody is a bispecific antibody which inhibits the activity of human IL-34 and human CSF-1. In some embodiments, the method comprises administering an effective amount of any of the anti-IL-34 antibodies provided herein in conjunction with an antibody that binds to human CSF-1. In some embodiments, the activity of human IL-34 and human CSF-1 is inhibited by a bispecific
- 20 anti-IL-34 and anti-CSF1 antibody. In some embodiments, the inhibition of activity is by inhibiting the binding of human IL-34 to human CSF-1R, and inhibiting the binding of human CSF-1 and human CSF-1R.

- In some embodiments of any of the methods above, the myeloid pathogenic immunological disease is rheumatoid arthritis (RA), inflammatory bowel disease (e.g., Crohn's, ulcerative
- 25 colitis), multiple sclerosis, systemic lupus erythematosus, lupus nephritis, asthma, osteoporosis, Paget's disease, atherosclerosis, metabolic syndrome, type II diabetes, macrophage activated syndrome (MAS), vasculitis (giant cell arteritis, ANCA associated vasculitis), discoid lupus, sarcoidosis, graft versus host disease, LSDs (lysosomal storage diseases like but not limited to Cystinosis, Salic acid storage disorder, Gaucher disease),
- 30 Histiocytosis including but not limited to Rosai-Dorfman disease, Faisalabad histiocytosis, H syndrome, pigmented hypertrichosis with insulin dependent diabetes (PHID)s, vasculitis, myocardial infarction and graft versus host disease. In one embodiment, the vasculitis is microscopic polyarteritis, CNS vasculitis, necrotizing, cutaneous, or hypersensitivity



vasculitis, systemic necrotizing vasculitis, or ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS)). In another embodiment, the vasculitis is large vessel vasculitis or medium vessel vasculitis. In one embodiment the large vessel vasculitis is polymyalgia rheumatica or giant cell arteritis or Takayasu's arteritis. In one embodiment, the

5 medium vessel vasculitis is Kawasaki's disease of polyarteritis nodosa. In one specific embodiment, an antibody of this invention (e.g., IL-34, bispecific IL-34/CSF1 antibody or CSF1R antibody) is used to treat RA patients who inadequately respond to a disease-modifying antirheumatic drug (DMARD) therapy (DMARD-IR). In one further embodiment, the DMARD-IR patient has not been previously treated with an anti-TNF agent ("TNF

10 naïve"). In one embodiment, the DMARD is methotrexate. In one specific embodiment, an antibody of this invention (e.g., IL-34 antibody, bispecific IL-34/CSF1 antibody or CSF1R antibody) is used to treat RA patients who inadequately respond to a disease-modifying antirheumatic drug (DMARD) therapy (DMARD-IR). In one further embodiment, the DMARD-IR patient has not been previously treated with an anti-TNF agent ("TNF naïve").

15 In one specific embodiment, an antibody of this invention (e.g., IL-34, bispecific IL-34/CSF1 antibody or CSF1R antibody) is used to treat RA patients who inadequately respond to anti-TNF therapies (e.g., TNFR-Fc or anti-TNF antibodies). "). In one specific embodiment, an antibody of this invention (e.g., IL-34, bispecific IL-34/CSF1 antibody or CSF1R antibody) is used to treat patients suffering from a myeloid pathogenic immunological disease who

20 inadequately responds to anti-TNF therapies (e.g., including, but not limited to, TNFR-Fc, anti-TNF antibodies and small molecule inhibitors of TNF or a TNF receptor).

In another embodiment of this invention, the RA patient to be treated with a CSF1-R pathway inhibitor of this invention has a Myeloid subtype and/or Fibroid subtype of RA. In one embodiment, the invention provides a method of treating rheumatoid arthritis in an individual

25 suffering therefrom comprising administering a CSF1-R pathway inhibitor to a patient who has been determined to have a myeloid subtype and/or a fibroid subtype of RA. In one embodiment, the Myeloid or Fibroid subtype is determined by measuring the gene expression level or protein expression level of a myeloid subtype or fibroid subtype gene and determining whether the RA individual has a myeloid or a fibroid subtype of RA, wherein a

30 determination that an RA individual has a myeloid or a fibroid subtype of RA indicates that the RA individual is more likely to respond to a CSF1-R pathway inhibitor.

In one embodiment of this invention, the pharmacodynamic effect of an antibody of this invention could be measured by monitoring the reduction in the levels of nonclassical

(CD14+CD16++) monocytes and/or intermediate (CD14++CD16+) monocytes in the blood of a patient after treatment with the antibody.

Further provided herein are methods of inhibiting binding between human IL-34 and human CSF-1R in an individual comprising administering to the individual an effective amount of  
5 any of the antibodies provided herein.

Further provided herein are articles of manufacture comprising any of the antibodies provided herein. In some embodiments, the article of manufacture further comprises instructions for administering an effective amount of the antibody to an individual for treating a myeloid pathogenic immunological disease in the individual. Also provided herein are articles of

10 manufacture comprising any of the anti-IL-34 antibodies provided herein and further comprising an antibody that binds to human CSF-1. In some embodiments, the article of manufacture further comprises instructions for administering an effective amount of the anti-IL-34 antibody and the antibody that binds to human CSF-1 to an individual for treating a myeloid pathogenic immunological disease in the individual. In some embodiments, the

15 myeloid pathogenic immunological disease is rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, lupus nephritis, asthma, osteoporosis, Paget's disease, atherosclerosis, metabolic syndrome, type II diabetes, LSDs (lysosomal storage diseases like but not limited to Cystinosis, Salic acid storage disorder, Gaucher disease), Histiocytosis including but not limited to Rosai-Dorfman disease,

20 Faisalabad histiocytosis, H syndrome, pigmented hypertrichosis with insulin dependent diabetes (PHID).

This invention provides a method for diagnosing an RA patient to be treated with a CSF1-R pathway inhibitor comprising the step of measuring the gene expression level or protein

25 expression level of a myeloid subtype or fibroid subtype gene and determining whether the RA individual has a myeloid or a fibroid subtype of RA, wherein a determination that an RA individual has a myeloid or a fibroid subtype of RA indicates that the RA individual is more likely to respond to a CSF1-R pathway inhibitor. In one embodiment, method further

30 comprises the step of measuring the gene or protein expression level of IL-34 and/or CSF-1 in the patient. In one embodiment, the CSF-1 level is measured in a biological sample from the sera or synovial fluid of an RA patient. In another embodiment, the IL-34 level is measured in the sera, synovial fluid or tissue biopsy of an RA patient.

Also provided herein is a polypeptide comprising the first three IgG domains (i.e., the first, second, and third IgG from the N-terminus) of a CSF-1R, wherein the polypeptide does not

comprise other IgG domains from the CSF-1R. In some embodiments, the polypeptide further comprises a linker between the IgG domains. In some embodiments, the polypeptide further comprises one or more fusion partners (e.g., an Fc sequence). Also provided herein are a nucleic acid encoding the polypeptide, a vector comprising the nucleic acid, and a host cell comprising the nucleic acid. Also provided herein is a method of producing the polypeptide comprising culturing a host cell that produces the polypeptide. Also provided herein is a method for treating a myeloid pathogenic immunological disease described herein comprising administering to an individual an effective amount of the polypeptide. Also provided herein is an article of manufacture comprising the polypeptide described herein.

It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art.

### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the structure of the functional core of human IL-34. (A) Schematic representation of the human IL-34. A predicted N-linked glycosylation site is indicated with star. The conserved disulfide bridges in IL-34 sequences across species are shown as dashed lines. (B) hIL-34s is active in promoting human monocyte viability. (C) Ribbon representation of the dimeric human IL-34s structure. The termini and secondary structures are labeled (when visible). (D) Ribbon representation of the murine CSF-1 structure. The termini and secondary structures are labeled.

Figure 2 shows the biophysical characterization of hIL-34s and hCSF-1 interactions with two different hCSF-1Rs containing domains D1-D3 and D1-D5. (A) Analytical size exclusion chromatography analyses of hIL-34s, CSF-1R D1-D3 and D1-D5, and their corresponding complexes. Chromatograms are shown overlaid from independent runs as described in the methods and referenced to molecular weight standards. Inset: SDS-PAGE of samples derived from peak fractions shown on the right. (B) Isothermal titration calorimetric measurements of the binding of CSF-1R D1-D5 to hIL-34s (left), CSF-1R D1-D3 to hIL-34s (middle) and displacement of CSF-1R D1-D3 from hIL-34s by CSF-1R D1-D5 (right). (C) Calorimetric measurements of the binding of CSF-1R D1-D3 to hCSF-1 (left) and CSF-1R D1-D5 to hCSF-1 (right). (D) Summary of the proteins in the syringe and their concentrations, the proteins in the cell and their concentrations, the enthalpy change ( $\Delta H$ ), entropy change ( $\Delta S$ ), Gibbs free energy change ( $\Delta G$ ), binding affinity ( $K_D$ ) and stoichiometry ( $n$ ) derived from

analyses of the three ITC titration experiments shown in panels B and C. N.D. indicates not determinable due to steepness of the curve.

Figure 3 shows a comparison of the Site 1 and 2 interfaces for CSF-1R in complex with IL-34 and CSF-1. (A–D) Close-up views of site 1 and site 2 of the IL-34/CSF-1R (A, C) or CSF-1/CSF-1R (B, D) interfaces. Key cytokine receptor interacting residues are shown as sticks, hydrogen bonds are drawn as dashed lines, and secondary structure elements are marked on the ribbons and strands.

Figure 4A shows inhibition of IL-34 biological activity by YW404.33.56 Fab in the monocyte viability assay. Figure 4B shows a close up view of interactions of CDR-loops (H1-H3, L3) of YW404.33.56 Fab with hIL-34s (cartoon representation). Critical residues involved in the interface interactions are highlighted in stick models.

Figure 5 shows receptor contacting residues mapped onto the secondary structure of IL-34 (A) and CSF-1 (B). The Site1 and Site2 interfacial residues are highlighted by dotted oval.

Figure 6 shows a comparison of the human IL-34/CSF-1R (left), murine CSF-1/CSF-1R (middle, PDB 3EJJ) and SCF/Kit (right, PDB 2E9W) signaling complex structures. The dimeric four-helical bundle cytokines are shown as cartoons and semitransparent surfaces. Receptor ectodomains are rendered as ribbon representation or shown as ovals for CSF-1R D4 and D5. The ionic pairs those have been implicated in the receptor homotypic contacts of CSF-1R and Kit are shown as circle and annotated.

Figure 7. Sequence alignment of selected IL-34 mammalian homologs (Homo sapiens (SEQ ID NO:68); Macaca mulatta (SEQ ID NO:69); Canis lupus familiaris (SEQ ID NO:70); Ailuropoda melanoleuca (SEQ ID NO:71); Equus caballus (SEQ ID NO:72); Bos taurus (SEQ ID NO:73); Mus musculus (SEQ ID NO:74); Rattus norvegicus (SEQ ID NO:75); Consensus Sequence (SEQ ID NO:76)). Numbering and secondary structure is according to the human IL-34 (SEQ ID NO:68). Strictly conserved residues are shaded in dark grey and conserved residues in most of the sequences, as calculated by a similarity score, are boxed. IL-34 residues at site 1, site 2 and IL-34 dimerization interface are denoted by solid circles, circles and stars at the bottom, respectively. Triangles indicate the disulfide bond pairing and glycosylation site. The alignment figures were made using program ESPRIT (WorldWide Web at [esprit.ibcp.fr/ESPrift/ESPrift](http://esprit.ibcp.fr/ESPrift/ESPrift)).

Figure 8 shows neutralizing activity of anti-IL-34 Ab YW404.33 in the monocyte proliferation assay.

Figure 9 shows neutralizing activity of anti-IL-34 Abs YW404.1, YW404.6, YW404.33, YW405.1, YW405.3, YW406.1, YW406.93 (A) and Abs YW404.33, YW404.33.12 and YW404.33.56 at a concentration of mIL-34 of 100 ng/ml (B) in the monocyte proliferation assay.

- 5 Figure 10: Variable heavy (A) and light (B) chain sequences of anti-IL-34 Abs YW404.1, YW404.3, YW404.33, YW404.33.10, YW404.33.12, YW404.33.11, YW404.33.56, and YW404.33.93. Amino acid residues targeted for affinity-maturation for these antibodies are surrounded by a box. Figure 10A shows the VH amino acid sequences for 404.1 (SEQ ID NO:15), 404.6 (SEQ ID NO: 77), 405.3 (SEQ ID NO:25), 404.33 (SEQ ID NO:5), 404.33.10
- 10 (SEQ ID NO:7), 404.33.12 (SEQ ID NO:11), 404.33.11 (SEQ ID NO:9), 404.33.56 (SEQ ID NO:3), and 404.33.93 (SEQ ID NO:13). Figure 10B shows the VL amino acid sequences for 404.1 (SEQ ID NO:16), 404.6 (SEQ ID NO: 78), 405.3 (SEQ ID NO:26), 404.33 (SEQ ID NO:6), 404.33.10 (SEQ ID NO:8), 404.33.12 (SEQ ID NO:12), 404.33.11 (SEQ ID NO:10), 404.33.56 (SEQ ID NO:4), and 404.33.93 (SEQ ID NO:14). The heavy chain framework
- 15 region sequences between Kabat HVRs are FR1 sequence (SEQ ID NO:17), FR2 sequence (SEQ ID NO:18), FR3 (SEQ ID NO:19), and FR4 (SEQ ID NO:20) shown in Figure 10A. The light chain framework region sequences between Kabat HVRs are FR1 sequence (SEQ ID NO:21), FR2 sequence (SEQ ID NO:22), FR3 sequence (SEQ ID NO:23), and FR4 sequence (SEQ ID NO:24) shown in Figure 10B.
- 20 Figure 11 shows the histology score of Balb/c mice with dextran sulfate sodium (DSS) – induced inflammatory bowel disease (IBD) treated with either control antibody (anti-ragweed, a-RW), cyclosporine (CSA), anti-CSF-1 antibody (a-CSF-1), anti-IL-34 antibody (a-IL-34) or a combination of anti-CSF-1 antibody and anti-IL-34 antibody.
- Figure 12 shows that serum levels of IL-34 and CSF-1 were elevated in Balb/c mice with
- 25 DSS-induced IBD treated with control antibody (a-RW) compared to control mice.
- Figure 13 shows CSF-1 and IL-34 are expressed in serum, synovial fluid and tissue from rheumatoid arthritis patients.
- Figure 14 shows that CSF1/IL34 pathway is present in primary and secondary TNF-NR RA patients.
- 30 Figure 15 shows that the treatment of a combination of aCSF1+aIL34 matches TNFR1I-Fc inflammation inhibition and is superior in protecting bone erosions in mouse CIA (myeloid drivers)
- Figure 16 shows the dual blockade of CSF1 and IL-34 inhibits DSS colitis in a model.

Figure 17 shows that IL-34 is expressed in IBD colon but low/undetectable in serum

Figure 18 shows that there is no correlation of IL-34/CSF-1 and TNF $\alpha$  expression in synovial fluid from rheumatoid arthritis and osteoarthritis patients.

Figure 19 shows the shows the reduction of mouse myeloid cells (Mf and monocytes)

5 infiltrating joint synovia after only 7 days of anti-CSF1/IL-34 combination treatment.

## DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

Without being bound by theory, the combinatorial approach of inhibiting both IL-34 and CSF-1 directly to treat myeloid pathogenic immunological diseases is believed to be superior to directly targeting their receptor or either IL-34 and CSF-1 alone. Advantages to this  
10 approach are predicted to include, but are not limited to, any one or combination of the following, better pharmacokinetic properties, better safety profiles, better efficacy, better potency and a better therapeutic window based on the safety and efficacy considerations above.

## DEFINITIONS

- 15 The terms “anti-IL-34 antibody” and “an antibody that binds to IL-34” refer to an antibody that is capable of binding IL-34 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting IL-34. In some embodiments, the extent of binding of an anti-IL-34 antibody to an unrelated, non-IL-34 protein is less than about 10% of the binding of the antibody to IL-34 as measured, e.g., by a BIAcore assay or a BLI assay.
- 20 In some embodiments, an antibody that binds to IL-34 has a dissociation constant ( $K_d$ ) of  $\leq 1 \mu\text{M}$ ,  $\leq 500 \text{ nM}$ ,  $\leq 250 \text{ nM}$ ,  $\leq 100 \text{ nM}$ ,  $\leq 10 \text{ nM}$ ,  $\leq 1 \text{ nM}$ ,  $\leq 0.1 \text{ nM}$ ,  $\leq 0.01 \text{ nM}$ , or  $\leq 0.001 \text{ nM}$  (e.g.,  $10^{-8} \text{ M}$  or less, e.g., from  $10^{-8} \text{ M}$  to  $10^{-13} \text{ M}$ , e.g., from  $10^{-9} \text{ M}$  to  $10^{-13} \text{ M}$ ). In some embodiments, an anti-IL-34 antibody binds to an epitope of IL-34 that is conserved among IL-34 from different species.
- 25 The term “IL-34,” as used herein, refers to any native IL-34 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed IL-34 as well as any form of IL-34 that results from processing in the cell. The term also encompasses naturally occurring variants of IL-34, e.g., splice variants or allelic variants. The amino acid sequence  
30 of an exemplary human IL-34 is shown in SEQ ID NO:1. In some embodiments, the human IL-34 comprises the amino acid sequence shown in SEQ ID NO:1, wherein amino acid Q at position 81 is deleted.

1 MPRGFTWLRY LGIFLGVALG NEPLEMWPLT QNEECTVTGF LRDKLQYRSR  
 LQYMKHYFPI  
 61 NYKISVPYEG VFRIANVTRL QRAQVSEREL RYLWVLVSL S ATESVQDVLL  
 5 EGHPSWKYLQ  
 121 EVETLLLNQV QGLTDVEVSP KVESVLSLLN APGPNLKLVR PKALLDNCFR  
 VMELLYCSCC  
 181 KQSSVLNWQD CEVPSPQSCS PEPSLQYAAT QLYPPPPWSP SSPPHSTGSV  
 RPYRAQGEG  
 10 241 LP (SEQ ID NO:1) .

The terms “anti- CSF-1 antibody” and “an antibody that binds to CSF-1” refer to an antibody that is capable of binding CSF-1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CSF-1. In some embodiments, the extent of  
 15 binding of an anti- CSF-1 antibody to an unrelated, non- CSF-1 protein is less than about 10% of the binding of the antibody to CSF-1 as measured, e.g., by a BIACORE assay or a BLI assay. In some embodiments, an antibody that binds to CSF-1 has a dissociation constant (K<sub>d</sub>) of  $\leq 1\mu\text{M}$ ,  $\leq 500\text{ nM}$ ,  $\leq 250\text{ nM}$ ,  $\leq 100\text{ nM}$ ,  $\leq 10\text{ nM}$ ,  $\leq 1\text{ nM}$ ,  $\leq 0.1\text{ nM}$ ,  $\leq 0.01\text{ nM}$ , or  $\leq 0.001\text{ nM}$  (e.g.,  $10^{-8}\text{ M}$  or less, e.g., from  $10^{-8}\text{ M}$  to  $10^{-13}\text{ M}$ , e.g., from  $10^{-9}\text{ M}$  to  $10^{-13}\text{ M}$ ). In  
 20 some embodiments, an anti-CSF-1 antibody binds to an epitope of CSF-1 that is conserved among CSF-1 from different species.

The term “CSF-1,” as used herein, refers to any native CSF-1 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed CSF-1 as well as any  
 25 form of CSF-1 that results from processing in the cell. The term also encompasses naturally occurring variants of CSF-1, e.g., splice variants or allelic variants. An exemplary human CSF-1 is described in Takahashi et al., *Biochem. Biophys. Res. Commun.* 161 (2), 892-901 (1989).

The terms “anti- CSF-1R antibody” and “an antibody that binds to CSF-1R” refer to an  
 30 antibody that is capable of binding CSF-1R with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CSF-1R. In some embodiments, the extent of binding of an anti- CSF-1R antibody to an unrelated, non- CSF-1R protein is less than about 10% of the binding of the antibody to CSF-1R as measured, e.g., by a BIACORE assay or a BLI assay. In some embodiments, an antibody that binds to CSF-1R

has a dissociation constant (K<sub>d</sub>) of  $\leq 1\mu\text{M}$ ,  $\leq 500\text{ nM}$ ,  $\leq 250\text{ nM}$ ,  $\leq 100\text{ nM}$ ,  $\leq 10\text{ nM}$ ,  $\leq 1\text{ nM}$ ,  $\leq 0.1\text{ nM}$ ,  $\leq 0.01\text{ nM}$ , or  $\leq 0.001\text{ nM}$  (e.g.,  $10^{-8}\text{ M}$  or less, e.g., from  $10^{-8}\text{ M}$  to  $10^{-13}\text{ M}$ , e.g., from  $10^{-9}\text{ M}$  to  $10^{-13}\text{ M}$ ). In some embodiments, an anti-CSF-1R antibody binds to an epitope of CSF-1R that is conserved among IL-34 from different species.

- 5 The term “CSF-1R” or “CSF1R” as used herein, refers to any native CSF-1R from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed CSF-1R as well as any form of CSF-1R that results from processing in the cell. The term also encompasses naturally occurring variants of CSF-1R, e.g., splice variants or allelic variants.
- 10 The amino acid sequence of an exemplary human CSF-1R is shown in SEQ ID NO:2.

MGPGVLLLLL VATAWHGQGI PVIEPSVPEL VVKPGATVTL RCVGNGSVEW  
 DGPPSPHWTL  
 YSDGSSSILS TNNATFQNTG TYRCTEPGDP LGGSAAIHLY VKDPAWPWNV  
 15 LAQEVVVFED  
 QDALLPCLLT DPVLEAGVSL VRVRGRPLMR HTNYSFSPWH GFTIHRAKFI  
 QSQDYQCSAL  
 MGGRKVMISIS IRLKVQKVIP GPPALTLVPA ELVRIRGEAA QIVCSASSVD  
 VNFDVFLQHN  
 20 NTKLAIPQQS DFHNNRYQKV LTLNLDQVDF QHAGNYSCVA SNVQGKHSTS  
 MFFRVVESAY  
 LNLSSSEQNLI QEVTVGEGLN LKVMVEAYPG LQGFNWTYLG PFSDHQPEPK  
 LANATTKDITY  
 RHTFTLSLPR LKPSEAGRYS FLARNPGGWR ALTFELTRY PPEVSVIWTF  
 25 INSGTLLCA  
 ASGYPQPNVT WLQCSGHTDR CDEAQVLQVW DDPYPEVLSQ EPFHKVTVQS  
 LLTVETLEHN  
 QTYECRAHNS VGSGSWAFIP ISAGATHPP DEFLFTPVVV ACMSIMALLL  
 LLLLLLLYKY  
 30 KQKPKYQVRW KIIESYEGNS YTFIDPTQLP YNEKWEFPRN NLQFGKTLGA  
 GAFGKVVEAT  
 AFGLGKEDAV LKVAVKMLKS TAHADEKEAL MSELKIMSHL GQHENIVNLL  
 GACTHGGPVL  
 VITEYCCYGD LLNFLRRKAE AMLGPSLSPG QDPEGGVVDYK NIHLEKKYVR  
 35 RDSGFSSQGV



DTYVEMRPVS TSSNDSFSEQ DLDKEDGRPL ELRDLLHFSS QVAQGMAFLA  
SKNCIHRDVA

ARNVLLTNGH VAKIGDFGLA RDIMNDSNYI VKGNARLPVK WMAPESIFDC  
VYTVQSDVWS

5 YGILLWEIFS LGLNPYPGIL VNSKFYKLVK DGYQMAQPAF APKNIYSIMQ  
ACWALEPTHR

PTFQQICSFL QEQAQEDRRE RDYTNLPSSS RSGGSGSSSS ELEEESSEH  
LTCCEQGDIA

QPLLQPNNYQ FC (SEQ ID NO:2)

- 10 A therapeutic agent according to this invention includes an agent that can bind to the target identified herein above, such as a polypeptide(s) (e.g., an antibody, an immunoadhesin or a peptibody), an aptamer or a small molecule that can bind to a protein or a nucleic acid molecule that can bind to a nucleic acid molecule encoding a target identified herein (i.e., siRNA).
- 15 The term “CSF1-R pathway inhibitor” refers to a therapeutic agent that inhibits CSF1-R signaling. In one embodiment, the CSF1-R pathway inhibitor binds to CSF-1, IL-34, CSF1-R or CSF-1 and IL-34. In one embodiment, the agent that binds CSF-1, IL-34 or CSF-1 and IL-34 inhibits the binding of such protein(s) to CSF1-R. In another embodiment, the agent that binds CSF1-R inhibits the binding of CSF1-R to IL-34 and CSF-1. In one embodiment, a
- 20 reduction in kinase activity of CSF1-R indicates a reduction in CSF-1R signalling. In one embodiment, the CSF1-R pathway inhibitor is an antibody of this invention. In another embodiment, the CSF-1R pathway inhibitor is a small molecule inhibitor of CSF1-R. In another embodiment, the CSF1-R pathway inhibitor is a CSF1-R extracellular domain fused to an Fc.
- 25 The term “antibody” herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.
- The term “variable region” or “variable domain” refers to the domain of an antibody heavy or
- 30 light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al., *Kuby Immunology*, 6<sup>th</sup> ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer

antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

5 The term “hypervariable region” or “HVR,” as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops (“hypervariable loops”). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the “complementarity  
10 determining regions” (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. An HVR as used herein can comprise residues located within positions 24-36 (for L1), 46-56 (for L2), 89-97 (for L3), 26-35B (for H1), 47-65 (for H2), and 93-102 (for H3). For example, an HVR can include residues in positions described previously:

15 A) 24-34 (L1), 50-56 (L2), 89-97 (L3), 26-32 (H1), 52-56 (H2), and 95-102 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987);

B) 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3 (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991); and

20 C) 30-36 (L1), 46-55 (L2), 89-96 (L3), 30-35 (H1), 47-58 (H2), 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262:732-745 (1996).

Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

25 Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise “specificity determining residues,” or “SDRs,” which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-  
30 CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues

and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

“Framework” or “FR” refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

A “human consensus framework” is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In some embodiments, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra*. In some embodiments, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

An “acceptor human framework” for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

The “class” of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgA<sub>1</sub>, and IgA<sub>2</sub>. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ , respectively.

The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In some embodiments, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy

chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

“Native antibodies” refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequence of its constant domain.

The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen.

Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

The term “chimeric” antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

5 A “humanized” antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In some embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody  
10 constant region derived from a human antibody. A “humanized form” of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This  
15 definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds.

Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')<sub>2</sub>; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv); and  
20 multispecific antibodies formed from antibody fragments.

The terms “full length antibody,” “intact antibody,” and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

25 An “isolated” antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).  
30

An “affinity matured” antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess

such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

“Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K<sub>d</sub>). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

An “antibody that binds to the same epitope” as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

“Effector functions” refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor); and B cell activation.

A “naked antibody” refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

An “isolated” nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

“Isolated nucleic acid encoding an anti-IL-34 antibody” refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

“Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical

with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

Alignment for purposes of determining percent amino acid sequence identity can be achieved

in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software.

Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity

values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco,

California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction  $X/Y$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating

nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

5 The terms “host cell,” “host cell line,” and “host cell culture” are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell,  
10 but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical  
15 pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay  
20 development of a disease or to slow the progression of a disease.

An “individual” or “subject” is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In some embodiments, the individual or subject is a human.

25 The term “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical  
30 formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.



An “effective amount” of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

As is understood in the clinical context, an effective amount of a therapeutic agent (e.g., an antibody provided herein), drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition.

Thus, an “effective amount” may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

As used herein, “in conjunction with” refers to administration of one treatment modality in addition to another treatment modality. As such, “in conjunction with” refers to administration of one treatment modality before, during or after administration of the other treatment modality to the individual.

The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

“Inflammatory bowel disease” or “IBD” refers to the group of disorders that cause the intestines to become inflamed, generally manifested with symptoms including abdominal cramps and pain, diarrhea, weight loss and intestinal bleeding. The main forms of IBD are ulcerative colitis (UC) and Crohn's disease.

As used herein, “myeloid pathogenic immunological disease” refers to an inflammatory disease and/or an autoimmune disease with a myeloid pathogenic component.

As used herein, “DMARD” refers to a disease-modifying antirheumatic drug. Examples of DMARDs include adalimumab, chloroquine, hydroxychloroquine, sulfasalazine, methotrexate, leflunomide, azathioprine, D-penicillamine, gold salts (sodium aurothiomalate, aurofin), Gold (oral), Gold (intramuscular), minocycline, cyclosporine, etanercept, golimumab, infliximab, minocycline and ritixumab.

As used herein, “F1” refers to fibroblast-rich type 1 subtype, “F2” refers to fibroblast-rich type 2 subtype, “L” refers to lymphoid-rich subtype or lymphoid subtype, and “M” refers to myeloid-rich subtype or myeloid subtype. Collectively, these subtypes identify four molecular subtypes of rheumatoid arthritis patients based on gene expression analysis.

Collectively, F1 and F2 subtypes are referred to as the fibroid or “F” subtype. The L subtype

of RA patients generally have a gene expression pattern characteristic of B cell, plasma cell, T cell, and macrophage involvement and evidence of B and T cell activation, isotype switching, Ig secretion, and cytokine production. The Myeloid subtype of RA patients generally have a gene expression pattern characteristic of monocyte, macrophage, neutrophil and lymphocyte involvement and evidence of macrophage activation, phagocytosis, respiratory burst, T cell activation and cytokine production. The Fibroid subtype of RA patients generally have a gene expression pattern characteristic of fibroblast and osteoblast involvement and evidence of bone formation, growth and differentiation and vasculogenesis.

As used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly indicates otherwise. For example, reference to an “antibody” is a reference to from one to many antibodies, such as molar amounts, and includes equivalents thereof known to those skilled in the art, and so forth.

Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to “about X” includes description of “X.”

It is understood that aspect and variations of the invention described herein include “consisting” and/or “consisting essentially of” aspects and variations.

## COMPOSITIONS AND METHODS

The invention provides antibodies that bind to IL-34, bispecific antibodies with a first binding specificity for IL-34 and a second binding specificity for CSF-1 (further referred to herein as bispecific anti-IL-34/CSF-1 antibodies), and antibodies that bind to CSF-1R. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of myeloid pathogenic immunological diseases, including, but not limited to inflammatory bowel disease, rheumatoid arthritis and multiple sclerosis. In some embodiments, the anti-IL-34 antibodies bind to mammalian (e.g., human) IL-34. In some embodiments, the bispecific anti-IL-34/CSF-1 antibodies comprise a first binding specificity to a mammalian (e.g., human) IL-34 and a second binding specificity to a mammalian (e.g., human) CSF-1. In some embodiments, the anti-CSF-1R antibodies bind to mammalian (e.g., human) CSF-1R.

### Exemplary Anti-IL-34 and CSF-1R Antibodies

#### Anti-IL-34 antibodies

In one aspect, the invention provides isolated antibodies that bind to IL-34 (e.g., human IL-34). The anti-IL-34 antibodies described herein may have one or more of the following

characteristics: (i) inhibition of binding of IL-34 (e.g., human IL-34) to CSF-1R (e.g., human CSF-1R); (ii) neutralization of IL-34 activity (e.g., human IL-34 activity); (iii) inhibition of IL-34 induced proliferation of peripheral blood mononuclear cells; (iv) binding to a dimer of IL-34 (e.g., human IL-34); (v) binding to an epitope that spans over both protomers of IL-34 (e.g., human IL-34); (vi) no inhibition of binding of CSF-1 (e.g., human CSF-1) to CSF-1R (e.g., human CSF-1R). In some embodiments, the extent of binding of an anti-IL-34 antibody to an unrelated, non-IL-34 protein is less than about 10% of the binding of the antibody to IL-34 as measured, e.g., by a BIACORE assay or a biolayer interferometry (BLI) assay. In some embodiments, the antibody that binds to IL-34 has a dissociation constant ( $K_d$ ) of  $\leq 1\mu\text{M}$ ,  $\leq 500\text{ nM}$ ,  $\leq 250\text{ nM}$ ,  $\leq 100\text{ nM}$ ,  $\leq 10\text{ nM}$ ,  $\leq 1\text{ nM}$ ,  $\leq 0.1\text{ nM}$ ,  $\leq 0.01\text{ nM}$ , or  $\leq 0.001\text{ nM}$  (e.g.,  $10^{-8}\text{ M}$  or less, e.g., from  $10^{-8}\text{ M}$  to  $10^{-13}\text{ M}$ , e.g., from  $10^{-9}\text{ M}$  to  $10^{-13}\text{ M}$ ). In some embodiments, the anti-IL-34 antibody has a  $K_d$  value of less than about 500 nM. In some embodiments, the anti-IL-34 antibody has a  $K_d$  value of less than about 100 nM or 10 nM. In some embodiments, the anti-IL-34 antibody has a  $K_d$  value of less than about 1 nM. In some embodiments, the IL-34 antibody has a  $K_d$  value of less than about 100 pM. In some embodiments, an anti-IL-34 antibody has a  $K_d$  of about 100-200 pM, about 100-500 pM, about 100 pM-1 nM, or of about 1nM-50nM. In some embodiments, an anti-IL-34 antibody has a  $K_d$  of about 17 nM. In some embodiments, an anti-IL-34 antibody has a  $K_d$  of about 120 nM. In some embodiments, the anti-IL-34 antibody binds to an epitope of IL-34 that is conserved among IL-34 from different species.

In one aspect, provided herein is an anti-IL-34 antibody, which binds to an epitope comprising at least any one of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, or sixteen, or seventeen of amino acid residues Glu103, Leu109, Gln106, Asn150, Leu127, Asn128, Ser184, Leu186, Asn187, Lys44, Glu121, Asp107, Glu111, Ser104, Gln120, Trp116, and Asn61 of a human IL-34. In one aspect, provided herein is an anti-IL-34 antibody, which binds to an epitope comprising at least one of amino acid residues from Glu103 to Asn150 of a human IL-34. In one aspect, provided herein is an anti-IL-34 antibody, which binds to an epitope comprising at least any one of one, two, or three, or four of amino acid residues Glu103, Leu109, Gln106, and Asn150 of a human IL-34. In any of the aspects above, the anti-IL-34 antibody may bind to an epitope further comprising at least any one of one, two, three, four, five, six, or seven, or eight of amino acid residues Ser100, Glu123, Trp116, Thr124, Leu127, Asn128, Gln131, and Thr134 of a human IL-34. In some embodiments, the anti-IL-34 antibody binds to amino acids within

positions 100-108, 116-134, 109 and 150 of a human IL-34. In some embodiments, the anti-IL-34 antibody inhibits binding between human IL-34 and human CSF-1R. In some embodiments, the anti-IL-34 antibody neutralizes human IL-34 activity. In some embodiments, the anti-IL-34 antibody binds to a dimer of human IL-34. In some

embodiments, the anti-IL-34 antibody binds to an epitope that spans both protomers of human IL-34. In some embodiments, the anti-IL-34 antibody is a monoclonal antibody. In some embodiments, the anti-IL-34 antibody is a human, humanized, or chimeric antibody. In some embodiments, the anti-IL-34 antibody is an antibody fragment that binds to human IL-34. As used herein, the residue position herein corresponds to the residue position in SEQ ID NO:1.

In one aspect, provided herein is an anti-IL-34 antibody, which binds to an epitope comprising at least any one of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, or sixteen, or seventeen of amino acid residues Glu103, Leu109, Gln106, Asn150, Leu127, Asn128, Ser184, Leu186, Asn187, Lys44, Glu121, Asp107, Glu111, Ser104, Gln120, Trp116, and Asn61 of a human IL-34. In one aspect,

provided herein is an anti-IL-34 antibody, which binds to an epitope comprising at least any one of one, two, three, four, or five, or six of amino acid residues Asn128, Ser184, Leu186, Asn187, Lys44, and Glu121 of a human IL-34. In any of the aspects above, the anti-IL-34 antibody may bind to an epitope further comprising at least any one of one, two, three, four, or five, or six of amino acid residues Phe40, Asp43, Leu125, Gln189, Thr36, and Val185 of a

human IL-34. In some embodiments, the anti-IL-34 antibody binds to amino acids within positions 36-44, 121-128, and 184-187 of a human IL-34. In some embodiments, the anti-IL-34 antibody inhibits binding between human IL-34 and human CSF-1R. In some embodiments, the anti-IL-34 antibody neutralizes human IL-34 activity. In some embodiments, the anti-IL-34 antibody binds to a dimer of human IL-34. In some

embodiments, the anti-IL-34 antibody binds to an epitope that spans both protomers of human IL-34. In some embodiments, the anti-IL-34 antibody is a monoclonal antibody. In some embodiments, the anti-IL-34 antibody is a human, humanized, or chimeric antibody. In some embodiments, the anti-IL-34 antibody is an antibody fragment that binds to human IL-34. As used herein, the residue position herein corresponds to the residue position in SEQ ID NO:1.

In one aspect, provided herein is an anti-IL-34 antibody that binds to an epitope comprising at least one of amino acid residues from Glu103-Leu127 of a human IL-34. In one aspect, provided herein is an anti-IL-34 antibody that binds to an epitope comprising at least any one of one, two, three, four, five, six, or seven, or eight of amino acid residues Asp107, Glu111,

Ser104, Gln120, Glu103, Leu109, Trp116, and Asn61 of a human IL-34. In any of the aspects provided above, the antibody may bind to an epitope which further comprises at least any one of one, two, three, four, five, six, seven, or eight, or nine of amino acid residues Pro152, Val108, Leu110, Gln106, Glu123, Leu127, Lys117, Ile60 and Lys55 of a human IL-34. In some embodiments, the antibody binds to amino acids within positions 55-61, 100-108, 109, 111-127 and 152 of a human IL-34. In some embodiments, the anti-IL-34 antibody inhibits binding between human IL-34 and human CSF-1R. In some embodiments, the anti-IL-34 antibody neutralizes human IL-34 activity. In some embodiments, the anti-IL-34 antibody binds to a dimer of human IL-34. In some embodiments, the anti-IL-34 antibody binds to an epitope that spans both protomers of human IL-34. In some embodiments, the anti-IL-34 antibody is a monoclonal antibody. In some embodiments, the anti-IL-34 antibody is a human, humanized, or chimeric antibody. In some embodiments, the anti-IL-34 antibody is an antibody fragment that binds to human IL-34. As used herein, the residue position herein corresponds to the residue position in SEQ ID NO:1.

In one aspect, the invention provides an anti-IL-34 antibody comprising at least any one of one, two, three, four, or five, or six HVRs in any combination as shown in Figures 10A and 10B. In some embodiments, the anti-IL-34 antibody comprises at least any one of one, two, three, four, or five, or six HVRs selected from (a) HVR-H1 comprising an amino acid sequence of STWIIH (SEQ ID NO: 59); (b) HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52); (c) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); (d) HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (e) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (f) HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39). In some embodiments, the anti-IL-34 antibody comprises at least any one of one, two, three, four, or five, or six HVRs selected from (a) HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59) or GFTFSST (SEQ ID NO: 30) or SSTWIIH (SEQ ID NO: 57), (b) HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52) or PYYYYY (SEQ ID NO: 37) or WVARISPYYYYSD (SEQ ID NO: 62); (c) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33) or ARGLGKGSKRGAMD (SEQ ID NO: 28); (d) HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50) or STAVAWY (SEQ ID NO: 58); (e) HVR-L2 comprising an amino acid sequence SASFLYS

(SEQ ID NO: 53) or LLIYSASFLY (SEQ ID NO: 34); and (f) HVR-L3 comprising an amino acid sequence of QQSFYFPNT (SEQ ID NO: 39) or QQSFYFPN (SEQ ID NO: 38).

In some embodiments, the anti-IL-34 antibody comprises at least any one of one, two, three, four, or five, or six HVRs selected from (a) HVR-H1 comprising an amino acid sequence of

- 5 STWIIH (SEQ ID NO: 59); (b) HVR-H2 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ ID NO: 51); (c) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); (d) HVR-L1 comprising an amino acid sequence of RASQDVSTAVA (SEQ ID NO: 50); (e) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (f) HVR-L3 comprising an amino acid sequence
- 10 QQYSDLPYT (SEQ ID NO: 45). In some embodiments, the anti-IL-34 antibody comprises at least any one of one, two, three, four, or five, or six HVRs selected from (a) HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59) or GFTFSST (SEQ ID NO: 30) or SSTWIIH (SEQ ID NO: 57); (b) HVR-H2 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ ID NO: 51) or PYSYG (SEQ ID NO: 36) or
- 15 WVARISPYSGYTN (SEQ ID NO: 61); (c) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33) or ARGLGKGSKRGAMD (SEQ ID NO: 28); (d) HVR-L1 comprising an amino acid sequence of RASQDVSTAVA (SEQ ID NO: 50) or STAVAWY (SEQ ID NO: 58); (e) HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 53) or LLIYSASFLY (SEQ ID NO: 34); and (f) HVR-L3
- 20 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45) or QQYSDLPY (SEQ ID NO: 44).

In some embodiments, the anti-IL-34 antibody comprises at least any one of one, two, three, four, five, or six HVRs selected from (a) a HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59); (b) a HVR-H2 comprising an amino acid sequence

- 25 RISPYYYYSDYADSVKG (SEQ ID NO: 52) or RISPYSGYTNYADSVKG (SEQ ID NO: 51); (c) a HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33) or GINQGSKRGAMDY (SEQ ID NO: 32); (d) a HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (e) a HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (f) a HVR-L3 comprising an amino acid
- 30 sequence QQSFYFPNT (SEQ ID NO: 39) or QQSYTTPPT (SEQ ID NO: 43) or QQYTALPYT (SEQ ID NO: 49) or QQYSDLPYT (SEQ ID NO: 45) or QQYSDVPYT (SEQ ID NO: 47) or QQSRTARPT (SEQ ID NO: 41). In some embodiments, the anti-IL-34 antibody comprises (a) a HVR-H3 comprising an amino acid sequence

GLGKGSKRGAMDY (SEQ ID NO: 33) or GINQGSKRGAMDY (SEQ ID NO: 32); (b) a HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39) or QQSYTTPPT (SEQ ID NO: 43) or QQYTALPYT (SEQ ID NO: 49) or QQYSDLPYT (SEQ ID NO: 45) or QQYSDVPYT (SEQ ID NO: 47) or QQSRTARPT (SEQ ID NO: 41); and (c) a HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52) or RISPYSGYTNYADSVKG (SEQ ID NO: 51). In some embodiments, the anti-IL-34 antibody comprises at least any one of one, two, three, four, or five, or six HVRs selected from (a) HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59) or GFTFSST (SEQ ID NO: 30) or SSTWIIH (SEQ ID NO: 57); (b) HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52) or RISPYSGYTNYADSVKG (SEQ ID NO: 51) or PYYYY (SEQ ID NO: 37) or PYSGY (SEQ ID NO: 36) or WVARISPYYYYSD (SEQ ID NO: 62) or WVARISPYSGYTN (SEQ ID NO: 61); and (c) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33) or GINQGSKRGAMDY (SEQ ID NO: 32) or ARGLGKGSKRGAMD (SEQ ID NO: 28) or ARGINQGSKRGAMD (SEQ ID NO: 27); (d) HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50) or STAVAWY (SEQ ID NO: 58); (e) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53) or LLIYSASFLY (SEQ ID NO: 34); and (f) HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39) or QQSYTTPPT (SEQ ID NO: 43) or QQYTALPYT (SEQ ID NO: 49) or QQYSDLPYT (SEQ ID NO: 45) or QQYSDVPYT (SEQ ID NO: 47) or QQSRTARPT (SEQ ID NO: 41) or QQSFYFPN (SEQ ID NO: 38) or QQSYTTPP (SEQ ID NO: 42) or QQYTALPY (SEQ ID NO: 48) or QQYSDLPY (SEQ ID NO: 44) or QQYSDVPY (SEQ ID NO: 46) or QQSRTARP (SEQ ID NO: 40).

In some embodiments, the anti-IL-34 antibody comprises at least any one of one, two, three, four, or five, or six HVRs selected from (a) a HVR-H1 comprising an amino acid sequence SNYIH (SEQ ID NO: 55); (b) a HVR-H2 comprising an amino acid sequence SITPASGDTDYADSVKG (SEQ ID NO: 54); (c) a HVR-H3 comprising an amino acid sequence SRGAYRFAY (SEQ ID NO: 56); (d) a HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (e) a HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (f) a HVR-L3 comprising an amino acid sequence QQSYTTPPT (SEQ ID NO: 43). In some embodiments, the anti-IL-34 antibody comprises (a) a HVR-H3 comprising an amino acid sequence SRGAYRFAY (SEQ ID NO: 56); (b) a HVR-L3 comprising an amino acid sequence QQSYTTPPT (SEQ ID NO: 43); and

(c) a HVR-H2 comprising an amino acid sequence SITPASGDDTDYADSVKG (SEQ ID NO: 54). In some embodiments, the anti-IL-34 antibody comprises at least any one of one, two, three, four, or five, or six HVRs selected from (a) HVR-H1 comprising an amino acid sequence SNYIH (SEQ ID NO: 55) or GFTFTSN (SEQ ID NO: 31) or TSNYIH (SEQ ID NO: 60); (b) HVR-H2 comprising an amino acid sequence SITPASGDDTDYADSVKG (SEQ ID NO: 54) or PASGD (SEQ ID NO: 35) or WVASITPASGDDTD (SEQ ID NO: 63); (c) HVR-H3 comprising an amino acid sequence SRGAYRFAY (SEQ ID NO: 56) or ARSRGAYRFA (SEQ ID NO: 29); (d) HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50) or STAVAWY (SEQ ID NO: 58); (e) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53) or LLIYSASFLY (SEQ ID NO: 34); and (f) HVR-L3 comprising an amino acid sequence QQSYTTPPT (SEQ ID NO: 43) or QQSYTTPP (SEQ ID NO: 42).

In one aspect, the invention provides an anti-IL-34 antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59); (b) HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52); (c) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33). In some embodiments, the anti-IL-34 antibody comprises HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33). In some embodiments, the anti-IL-34 antibody comprises (a) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33), and (b) a HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39). In some embodiments, the anti-IL-34 antibody comprises (a) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); (b) HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39); and (c) HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52). In some embodiments, the antibody comprises (a) HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59); (b) HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52); and (c) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33).

In another aspect, the invention provides an anti-IL-34 antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (b) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) HVR-L3 comprising an amino acid sequence



QQSFYFPNT (SEQ ID NO: 39). In some embodiments, the antibody comprises (a) HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (b) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39).

- 5 In another aspect, an anti-IL-34 antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59), (ii) HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52), and (iii) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); and (b) a VL
- 10 domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50), (ii) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53), and (iii) HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39).

- In another aspect, the invention provides an anti-IL-34 antibody comprising (a) HVR-H1
- 15 comprising an amino acid sequence of STWIIH (SEQ ID NO: 59); (b) HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52); (c) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); (d) HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (e) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (f) HVR-L3 comprising an amino
- 20 acid sequence QQSFYFPNT (SEQ ID NO: 39).

- In one aspect, the invention provides an anti-IL-34 antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59); (b) HVR-H2 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ ID NO: 51); (c) HVR-H3 comprising an amino acid
- 25 sequence GLGKGSKRGAMDY (SEQ ID NO: 33). In some embodiments, the anti-IL-34 antibody comprises HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33). In some embodiments, the anti-IL-34 antibody comprises (a) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33), and (b) a HVR-L3 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45). In some
- 30 embodiments, the anti-IL-34 antibody comprises (a) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); (b) HVR-L3 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45); and (c) HVR-H2 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ ID NO: 51). In some embodiments, the antibody

comprises (a) HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59); (b) HVR-H2 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ ID NO: 51); and (c) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33).

- 5 In another aspect, the invention provides an anti-IL-34 antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising an amino acid sequence of RASQDVSTAVA (SEQ ID NO: 50); (b) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) HVR-L3 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45). In some embodiments, the antibody comprises
- 10 (a) HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (b) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) HVR-L3 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45).

- In another aspect, an anti-IL-34 antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-
- 15 H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59), (ii) HVR-H2 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ ID NO: 51), (iii) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising an amino acid sequence of RASQDVSTAVA (SEQ ID NO: 50); (ii)
- 20 HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (iii) HVR-L3 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45).

- In another aspect, the invention provides an anti-IL-34 antibody comprising (a) HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59); (b) HVR-H2 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ ID NO: 51); (c) HVR-H3 comprising
- 25 an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); (d) HVR-L1 comprising an amino acid sequence of RASQDVSTAVA (SEQ ID NO: 50); (e) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (f) HVR-L3 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45).

- In another aspect, the invention provides an anti-IL-34 antibody comprising (a) HVR-H1
- 30 comprising an amino acid sequence SNWIIH (SEQ ID NO: 79), (b) HVR-H2 comprising an amino acid sequence RISPNSGYTDYADSVKG (SEQ ID NO: 80); (c) HVR-H3 comprising an amino acid sequence SMRARRGFDY (SEQ ID NO: 81); (d) HVR-L1 comprising an amino acid sequence of RASQDVSTAVA (SEQ ID NO: 50); (e) HVR-L2 comprising an

amino acid sequence SASFLYS (SEQ ID NO: 53); and (f) HVR-L3 comprising an amino acid sequence QQSYTTPPT (SEQ ID NO: 43).

In another aspect, the invention provides an anti-IL-34 antibody derived from an anti-IL-34 antibody exemplified herein.

- 5 In some embodiments, the anti-IL-34 antibody comprises any one or any combination of two, three, four, five, or six of the following HVRs:

HVR-H1:  $SX_1X_2IH$ , wherein  $X_1$  is N or T, and  $X_2$  is Y or W (SEQ ID NO: 64);

HVR-H2:  $X_1IX_2PX_3X_4X_5X_6X_7X_8YADSVKG$ , wherein  $X_1$  is S or R; and  $X_2$  is T or S;  $X_3$  is A or Y;  $X_4$  is S or Y;  $X_5$  is G or Y;  $X_6$  is D or Y;  $X_7$  is T or S; and  $X_8$  is D or N (SEQ ID NO: 65);

HVR-H3: SRGAYRFAY (SEQ ID NO: 56), or  $GX_1X_2X_3GSKRGAMDY$ , wherein  $X_1$  is L or I;  $X_2$  is G or N;  $X_3$  is K or Q (SEQ ID NO: 66);

HVR-L1: RASQDVSTAVA (SEQ ID NO: 50);

HVR-L2: SASFLYS (SEQ ID NO: 53);

- 15 HVR-L3:  $QQX_1IX_2PX_3X_4X_5X_6T$ , wherein the  $X_1$  is S or Y; and  $X_2$  is Y, T, S, F, or R;  $X_3$  is T, A, D, or Y;  $X_4$  is T, L, V, F, or A;  $X_5$  is P or R;  $X_6$  is P, Y, or N (SEQ ID NO: 67).

In some embodiments, one or more amino acid residues in HVRs may be substituted. In some embodiments, the substitutions are conservative substitutions, as provided herein.

- In any of the above embodiments, an anti-IL-34 antibody is humanized. In some  
20 embodiments, an anti-IL-34 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g., a human immunoglobulin framework or a human consensus framework. In another embodiment, an anti-IL-34 antibody comprises HVRs as in any of the above embodiments, and further comprises a VH comprising an FR1 sequence of SEQ ID NO:17, an FR2 sequence of SEQ ID NO:18, an FR3  
25 sequence of SEQ ID NO:19, a FR4 sequence of SEQ ID NO:20 and/or a VL comprising an FR1 sequence of SEQ ID NO:21, an FR2 sequence of SEQ ID NO:22, an FR3 sequence of SEQ ID NO:23, a FR4 sequence of SEQ ID NO:24.

- In another aspect, an anti-IL-34 antibody comprises a heavy chain variable domain (VH) sequence having at least any one of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or  
30 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:3 (VH amino acid sequence of antibody 404.33.56 shown Figure 10A). In some embodiments, a VH sequence having at least any one of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions

relative to the reference sequence, but an anti-IL-34 antibody comprising that sequence retains the ability to bind to IL-34. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:3. In some embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

5 Optionally, the anti-IL-34 antibody comprises the VH sequence in SEQ ID NO:3, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising an amino acid sequence STWIH (SEQ ID NO: 59); (b) HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52); (c) HVR-H3 comprising an amino acid  
10 sequence GLGKGSKRGAMDY (SEQ ID NO: 33).

In another aspect, an anti-IL-34 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least any one of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:4 (VL amino acid sequence of antibody 404.33.56 shown Figure 10B). In some

15 embodiments, a VL sequence having at least any one of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-34 antibody comprising that sequence retains the ability to bind to IL-34. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:4. In some  
20 embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-IL-34 antibody comprises the VL sequence in SEQ ID NO:4, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (b) HVR-L2  
25 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) HVR-L3 comprising an amino acid sequence QQSIFYFPNT (SEQ ID NO: 39).

In another aspect, an anti-IL-34 antibody comprises a heavy chain variable domain (VH) sequence having at least any one of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:11 (VH amino  
30 acid sequence of antibody 404.33.12 shown Figure 10A). In some embodiments, a VH sequence having at least any one of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-34 antibody comprising that sequence

retains the ability to bind to IL-34. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:11. In some embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

Optionally, the anti-IL-34 antibody comprises the VH sequence in SEQ ID NO:11, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising an amino acid sequence of STWIIH (SEQ ID NO: 59); (b) HVR-H2 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ ID NO: 51); (c) HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33).

In another aspect, an anti-IL-34 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least any one of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:12 (VL amino acid sequence of antibody 404.33.12 shown Figure 10B). In some embodiments, a VL sequence having at least any one of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-34 antibody comprising that sequence retains the ability to bind to IL-34. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:12. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-IL-34 antibody comprises the VL sequence in SEQ ID NO:12, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising an amino acid sequence of RASQDVSTAVA (SEQ ID NO: 50); (b) HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) HVR-L3 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45).

In another aspect, an anti-IL-34 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO:3 and SEQ ID NO:4, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 11 and SEQ ID NO:12, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO:5 and SEQ ID NO:6, respectively, including post-translational modifications

of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 7 and SEQ ID NO:8, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 9 and SEQ ID NO:10, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO:13 and SEQ ID NO:14, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO:15 and SEQ ID NO:16, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO:77 and SEQ ID NO:78, respectively, including post-translational modifications of those sequences.

In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-IL-34 antibody provided herein. For example, in some embodiments, an antibody is provided that binds to the same epitope as an anti-IL-34 antibody selected from the of an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:3 and a VL sequence of SEQ ID NO:4, an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:11 and a VL sequence of SEQ ID NO:12, an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:5 and a VL sequence of SEQ ID NO:6, an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8, an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:9 and a VL sequence of SEQ ID NO:10, an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:13 and a VL sequence of SEQ ID NO:14, or an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:16. In some embodiments, the anti-IL-34 antibody binds to the same epitope as an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:3 and a VL sequence of SEQ ID NO:4. In some embodiments, the anti-IL-34 antibody binds to the same epitope as an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:11 and a VL sequence of SEQ ID NO:12. In some embodiments, the epitope is a conformational epitope. In some embodiments, the anti-IL-34 antibody binds to the same epitope as an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:77 and a VL sequence of SEQ ID NO:78. In some embodiments, the epitope is a conformational epitope. In some embodiments, the epitope is a linear epitope.

In a further aspect of the invention, an anti-IL-34 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody.

In some embodiments, an anti-IL-34 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')<sub>2</sub> fragment. In another embodiment, the antibody is a full length antibody, e.g., an intact IgG1 or IgG4 antibody or other antibody class or isotype as defined herein.

- 5 In a further aspect, an anti-IL-34 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

#### Anti-CSF-1R antibodies

In another aspect, the invention provides isolated antibodies that bind to CSF-1R (e.g., human  
10 CSF-1R). In one aspect, provided herein is an anti-CSF-1R antibody, which binds to an epitope comprising at least any one of one, two, three, four, or five, or six of amino acid residues Arg144, Gln248, Gln249, Ser250, Phe252, and Asn254 of human CSF-1R. In one aspect, provided herein is an anti-CSF-1R antibody, which binds to an epitope comprising amino acid residue Arg144 of human CSF-1R. In one aspect, provided herein is an anti-CSF-  
15 1R antibody, which binds to an epitope comprising at least any one of one, two, or three, or four of amino acid residues Arg144, Arg142, Arg146, and Arg250 of human CSF-1R. The anti-CSF-1R antibody of any of the aspects above may bind to an epitope further comprising at least one, or two of amino acid residues Ser172 and Arg192 of human CSF-1R. The anti-CSF-1R antibody of any of the aspects above may bind to an epitope further comprising at  
20 least any one of one, two, three, four, or five, or six of amino acid residues Arg146, Met149, Arg150, Phe169, Ile170, and Gln173 of human CSF-1R. In some embodiments, the anti-CSF-1R antibody binds to amino acids within positions 142-150 and 169-172 of CSF-1R. As used herein, the residue position herein corresponds to the residue position in SEQ ID NO:2. In some embodiments, the anti-CSF-1R antibody inhibits binding between human IL-34 and/or  
25 human CSF-1 to human CSF-1R.

In another aspect, provided herein is an anti-CSF-1R antibody, which binds to an epitope comprising at least any one of one, two, three, four, or five, or six of amino acid residues Arg144, Gln248, Gln249, Ser250, Phe252, and Asn 254 of human CSF-1R. In one aspect, provided herein is an anti-CSF-1R antibody, which binds to an epitope comprising at least  
30 any one of one, two, three, or four, or five of amino acid residues Gln248, Gln249, Ser250, Phe252, and Asn254 of human CSF-1R. In one aspect, provided herein is an anti-CSF-1R antibody, which binds to an epitope comprising at least any one of one, two, three, four, or five, or six of amino acid residues Gln248, Gln249, Ser250, Phe252, Asn254, and Tyr257 of

human CSF-1R. The anti-CSF-1R antibody of any of the aspects above may bind to an epitope further comprising at least one, at least two, or three of amino acid residues Pro247, Gln258, and Lys259 of human CSF-1R. The anti-CSF-1R antibody of any of the aspects above may bind to an epitope further comprising at least one, at least two, or three of amino acid residues Val231, Asp251, and Tyr257 of human CSF-1R. In some embodiments, the anti-CSF-1R antibody binds to amino acids within positions 231, 248-252 and 254 of CSF-1R. As used herein, the residue position herein corresponds to the residue position in SEQ ID NO:2. In some embodiments, the anti-CSF-1R antibody inhibits binding between human IL-34 and/or human CSF-1 to human CSF-1R.

In a further aspect of the invention, an anti-CSF1R antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In some embodiments, anti-CSF-1R antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')<sub>2</sub> fragment. In another embodiment, the antibody is a full length antibody, e.g., an intact IgG1 or IgG4 antibody or other antibody class or isotype as defined herein.

In a further aspect, an anti-CSF-1R antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

### ***Antibody Affinity***

In some embodiments, an antibody provided herein has a dissociation constant (K<sub>d</sub>) of  $\leq 1\mu\text{M}$ ,  $\leq 100\text{ nM}$ ,  $\leq 10\text{ nM}$ ,  $\leq 1\text{ nM}$ ,  $\leq 0.1\text{ nM}$ ,  $\leq 0.01\text{ nM}$ , or  $\leq 0.001\text{ nM}$  (e.g.,  $10^{-8}\text{ M}$  or less, e.g., from  $10^{-8}\text{ M}$  to  $10^{-13}\text{ M}$ , e.g., from  $10^{-9}\text{ M}$  to  $10^{-13}\text{ M}$ ).

In some embodiments, K<sub>d</sub> is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (<sup>125</sup>I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER<sup>®</sup> multi-well plates (Thermo Scientific) are coated overnight with 5  $\mu\text{g/ml}$  of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [<sup>125</sup>I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent



with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached.

Thereafter, the mixtures are transferred to the capture plate for incubation at room

temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20<sup>®</sup>) in PBS. When the plates have dried, 150  $\mu$ l/well of scintillant (MICROSCINT-20<sup>™</sup>; Packard) is added, and the plates are counted on a TOPCOUNT<sup>™</sup> gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

According to another embodiment, K<sub>d</sub> is measured using surface plasmon resonance assays using a BIACORE<sup>®</sup>-2000 or a BIACORE<sup>®</sup>-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N*'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5  $\mu$ g/ml (~0.2  $\mu$ M) before injection at a flow rate of 5  $\mu$ l/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20<sup>™</sup>) surfactant (PBST) at 25°C at a flow rate of approximately 25  $\mu$ l/min.

Association rates (k<sub>on</sub>) and dissociation rates (k<sub>off</sub>) are calculated using a simple one-to-one Langmuir binding model (BIACORE<sup>®</sup> Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K<sub>d</sub>) is calculated as the ratio k<sub>off</sub>/k<sub>on</sub>. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10<sup>6</sup> M<sup>-1</sup> s<sup>-1</sup> by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO<sup>™</sup> spectrophotometer (ThermoSpectronic) with a stirred cuvette.

According to another embodiment, the K<sub>d</sub> is measured using a BLI assay, for example, as described herein.

### *Antibody Fragments*

In some embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')<sub>2</sub>, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')<sub>2</sub> fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993).

Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In some embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 B1).

In some embodiments, an antibody fragment is a monovalent antibody that has an in vivo half life substantially similar to an intact antibody. For example, such an antibody fragment may comprise one antigen binding arm linked to an Fc sequence capable of conferring in vivo stability to the fragment. In one embodiment, an antibody of the invention is a one-armed antibody as described in WO2005/063816. In one embodiment, the one-armed antibody comprises Fc mutations constituting "knobs" and "holes" as described in WO2005/063816. The antibody fragment may also be a "linear antibody", e.g., as described in U.S. Pat. No. 5,641,870. Such linear antibody fragments may be monospecific or bispecific.

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g., *E. coli* or phage), as described herein.

### *Chimeric and Humanized Antibodies*

In some embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a “class switched” antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

In some embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat’l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing “resurfacing”); Dall’Acqua et al., *Methods* 36:43-60 (2005) (describing “FR shuffling”); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the “guided selection” approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the “best-fit” method (see, e.g., Sims et al., *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et

al., *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al., *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

### ***Human Antibodies***

In some embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM

antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical*  
 5 *Pharmacology*, 27(3):185-91 (2005).

Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

### 10 ***Library-Derived Antibodies***

Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in  
 15 Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132 (2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*,  
 25 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as  
 30 described by Griffiths et al., *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to

accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

## 6. Multispecific Antibodies

### Bispecific Antibodies

Bispecific antibodies are monoclonal antibodies that have binding specificities for two different antigens. In some embodiments, bispecific antibodies are human or humanized antibodies. In some embodiments, one of the binding specificities is for IL-34 (e.g., human IL-34) and the other is for any other antigen. In some embodiments, bispecific antibodies may bind to two different epitopes of IL-34 (e.g., human IL-34). In some embodiments, bispecific antibodies comprise a first binding specificity to IL-34 (e.g., human IL-34) and a second binding specificity to CSF-1 (e.g., human CSF-1). In some embodiments, bispecific antibodies bind to the same epitope on IL-34 as any of the anti-IL-34 antibodies described herein. In some embodiments, bispecific antibodies comprise at least any one of one, two, three, four, or five or six HVRs of any one of the anti-IL-34 antibodies described herein. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g., F(ab')<sub>2</sub> bispecific antibodies).

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, *Nature* 305: 537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 published May 13, 1993, and in Traunecker et al., *EMBOJ.*, 10: 3655 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion, for example, is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. In some embodiments, the first heavy-chain constant region (CH1), containing the site necessary for light chain binding, is present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In some embodiments of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 121:210 (1986).

According to another approach, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The interface comprises at least a part of the CH3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g., tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or “heteroconjugate” antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO

5 92/00373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking method. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques. Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical  
10 linkage. Brennan et al., *Science* 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')<sub>2</sub>' fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted  
15 to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.*, 175:  
20 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')<sub>2</sub> molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human  
25 breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of  
30 two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The “diabody” technology described by Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)



has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., *J. Immunol.*, 152:5368 (1994).

According to one embodiment, one polypeptide comprising an antigen binding domain of this invention comprises a heterodimerization domain. As used herein, “heteromultimerization domain” refers to alterations or additions to a biological molecule so as to promote heteromultimer formation and hinder homomultimer formation. Any heterodimerization domain having a strong preference for forming heterodimers over homodimers is within the scope of the invention. Illustrative examples include but are not limited to, for example, US Patent Application 20030078385 (Arathoon et al.; describing knob-into-holes); WO2007147901 (Kjærgaard et al.; describing ionic interactions); WO 2009089004 (Kannan et al.; describing electrostatic steering effects); WO2011/034605 (Christensen et al.; describing coiled coils). See also, for example, Pack, P. & Plueckthun, A., *Biochemistry* 31, 1579-1584 (1992) describing leucine zipper or Pack et al., *Bio/Technology* 11, 1271-1277 (1993) describing the helix-turn-helix motif. The phrase “heteromultimerization domain” and “heterodimerization domain” are used interchangeably herein.

The term “knob-into-hole” or “KnH” technology as mentioned herein refers to the technology directing the pairing of two polypeptides together in vitro or in vivo by introducing a pertuberance (knob) into one polypeptide and a cavity (hole) into the other polypeptide at an interface in which they interact. For example, KnHs have been introduced in the Fc:Fc binding interfaces, CL:CH1 interfaces or VH/VL interfaces of antibodies (e.g., US2007/0178552, WO 96/027011, WO 98/050431 and Zhu et al. (1997) *Protein Science* 6:781-788).

Further techniques for making multispecific, e.g., bispecific, antibodies include, but are not limited to, “knob-in-hole” engineering (see, e.g., U.S. Patent No. 5,731,168), engineering using electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., *J. Immunol.* 147: 60 (1991).

Engineered antibodies with three or more functional antigen binding sites, including “Octopus antibodies,” are also included herein (see, e.g., US 2006/0025576A1).

- 5 The antibody or fragment herein also includes a “Dual Acting FAb” or “DAF” comprising an antigen binding site that binds to IL-34 as well as another, different antigen (e.g., CSF-1) (see, US2008/0069820, for example).

### 7. *Antibody Variants*

- In some embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

#### **Substitution, Insertion, and Deletion Variants**

- In some embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of “conservative substitutions.” More substantial changes are provided in Table 1 under the heading of “exemplary substitutions,” and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

**TABLE 1**

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln

Original Residue	Exemplary Substitutions	Preferred Substitutions
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent

antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g., binding affinity).

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR “hotspots,” i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O’Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In some embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs.

Such alterations may be outside of HVR “hotspots” or SDRs. In some embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be

introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution.

- 5 Variants may be screened to determine whether they contain the desired properties. Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional
- 10 variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g., for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

#### **Glycosylation variants**

- In some embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.
- 15

- Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al., *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.
- 20
- 25

- In some embodiments, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e.g., complex, hybrid and high mannose structures) as measured by MALDI-TOF
- 30

mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about  $\pm 3$  amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence

5 variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., U.S. Patent Publication Nos. US 2003/0157108 (Presta, L.); U.S. 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to “defucosylated” or “fucose-deficient” antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US

10 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al., *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al., *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO

cells deficient in protein fucosylation (Ripka et al., *Arch. Biochem. Biophys.* 249:533-545

15 (1986); U.S. Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., *Acta crystallographica Section D, Biological crystallography* 66: 213-221 (2010), especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (see, e.g., Yamane-Ohnuki et al., *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

20 Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function.

Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); U.S. Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.).

25 Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

#### **Fc region variants**

30 In some embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4

Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions.

In some embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability.

The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g., Hellstrom, I. et al., *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)).

Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes et al., *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). Clq binding assays may also be carried out to confirm that the antibody is unable to bind Clq and hence lacks CDC activity. See, e.g., Clq and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265,

269, 270, 297 and 327, including the so-called “DANA” Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2):

6591-6604 (2001).)

In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) Clq binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in U.S. Patent No. 6,194,551, WO 99/51642, and Idusogie et al., *J. Immunol.* 164: 4178-4184 (2000).

Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (U.S. Patent No. 7,371,826).

See also Duncan et al., *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

#### **Cysteine engineered antibody variants**

In some embodiments, it may be desirable to create cysteine engineered antibodies, e.g., “thioMAbs,” in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In some embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region.

Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.



### Antibody Derivatives

In some embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide copolymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In some embodiments, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

### **Recombinant Methods and Compositions**

Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In some embodiments, isolated nucleic acid encoding an anti-IL-34 antibody, a bispecific anti-IL-34/CSF-1 antibody or an anti-CSF-1R antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or

heavy chains of the antibody). In some embodiments, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In some embodiments, a host cell comprising such nucleic acid is provided. In some embodiments, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In some embodiments, the host cell is eukaryotic, e.g., a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In some embodiments, a method of making an anti-IL-34 antibody, a bispecific anti-IL-34/CSF-1 antibody or an anti-CSF-1R antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

For recombinant production of an anti-IL-34 antibody, a bispecific anti-IL-34/CSF-1 antibody or an anti-CSF-1R antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an

antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures can also be utilized as hosts. See, e.g., U.S. Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES<sup>TM</sup> technology for producing antibodies in transgenic plants).

Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR<sup>-</sup> CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

### Assays

Anti-IL-34 antibodies, bispecific anti-IL-34/CSF-1 antibodies and anti-CSF-1R antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

#### *Binding assays and other assays*

In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

In another aspect, competition assays may be used to identify an anti-IL-34 antibody or a bispecific anti-IL-34/CSF-1 antibody that competes with, for example, an anti-IL-34 antibody described herein. For example, antibodies that compete with an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:5 and a VL sequence of SEQ ID NO:6 for binding to IL-34. In some embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by, for example, an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:5 and a VL sequence of SEQ ID NO:6. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

In an exemplary competition assay, immobilized IL-34 is incubated in a solution comprising a first labeled antibody that binds to IL-34 (e.g., an anti-IL-34 antibody comprising a VH sequence of SEQ ID NO:5 and a VL sequence of SEQ ID NO:6) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to IL-34. The second antibody may be present in a hybridoma supernatant. As a control, immobilized IL-34 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to IL-34, excess unbound antibody is removed, and the amount of label associated with immobilized IL-34 is measured. If the amount of label associated with immobilized IL-34 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to IL-34. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

In one aspect, assays are provided for identifying anti-IL-34 antibodies, bispecific anti-IL-34/CSF-1 antibodies or anti-CSF1R antibodies having biological activities. Biological activity may include, e.g., inhibition of proliferation of human peripheral blood mononuclear cells (PBMCs), inhibition of binding of IL-34 to CSF-1R, or inhibition of binding of CSF-1 to CSF-1R. Antibodies having such biological activity in vivo and/or in vitro are also provided.

In some embodiments, an antibody of the invention is tested for such biological activity. For example, the neutralizing activity of an anti-IL-34 antibody, a bispecific anti-IL-34/CSF-1 antibody or anti-CSF-1R antibody can be measured using a cell proliferation assay by CellTiter-Glo. hIL-34 or mIL-34 is combined with serial dilutions of anti-IL-34 mAbs,

bispecific anti-IL-34/CSF-1 antibodies or anti-CSF1 antibodies before adding onto cells, such as peripheral blood mononuclear cells (PBMCs). The antibody inhibition activity is obtained by measuring RLU after incubating the plates at 37 °C for 72 hours. The Half Maximal Inhibitory Concentration (IC50), defined as the concentration of antibody required to yield half maximal inhibition of IL-34 activity on cells, when IL-34 is present at a concentration to elicit 70-80% proliferation response, can be calculated with KaleidaGraph.

Inhibition of binding of IL-34 or CSF-1 to CSF-1R by an antibody provided herein may be tested in ELISA assays using immobilized IL-34 or CSF-1 and soluble CSF-1R in the presence of serial dilution of the antibody, e.g., an anti-IL-34 antibody, bispecific IL-34/CSF-1 antibody or anti-CSF-1 antibody.

### Methods and Compositions for Diagnostics and Detection

In some embodiments, any of the anti-IL-34 antibodies and anti-CSF-1R provided herein is useful for detecting the presence of IL-34 or CSF-1R in a biological sample. The term “detecting” as used herein encompasses quantitative or qualitative detection. In some embodiments, a biological sample comprises a cell or tissue.

In some embodiments, anti-IL-34 antibodies for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of IL-34 in a biological sample is provided. In some embodiments, the method comprises contacting the biological sample with an anti- IL-34 antibody as described herein under conditions permissive for binding of the anti- IL-34 antibody to IL-34, and detecting whether a complex is formed between the anti- IL-34 antibody and IL-34. Such method may be an *in vitro* or *in vivo* method. In some embodiments, an anti- IL-34 antibody is used to select subjects eligible for therapy with an anti-IL-34 antibody, e.g., where IL-34 is a biomarker for selection of patients. In some embodiments, anti-CSF-1R antibodies for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of CSF-1R in a biological sample is provided. In some embodiments, the method comprises contacting the biological sample with an anti- CSF-1R antibody as described herein under conditions permissive for binding of the anti-CSF-1R antibody to CSF-1R, and detecting whether a complex is formed between the anti-CSF-1R antibody and CSF-1R. Such method may be an *in vitro* or *in vivo* method. In some embodiments, an anti-CSF-1R antibody is used to select subjects eligible for therapy with an anti-CSF-1R antibody, e.g., where CSF-1R is a biomarker for selection of patients.

Exemplary disorders that may be diagnosed using an antibody of the invention include myeloid pathogenic immunological diseases such as rheumatoid arthritis, inflammatory bowel disease, or multiple sclerosis.

In some embodiments, labeled anti-IL-34 antibodies and anti-CSF-1R are provided. Labels  
 5 include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes  $^{32}\text{P}$ ,  $^{14}\text{C}$ ,  $^{125}\text{I}$ ,  $^3\text{H}$ , and  $^{131}\text{I}$ , fluorophores such as rare earth chelates or  
 10 fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase,  $\beta$ -galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as  
 15 uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

#### Biomarkers for Myeloid and Fibroid Subtypes

In one embodiment, RA patients to be treated with a CSF1-R pathway inhibitor are those  
 20 patients that correspond to the Myeloid or Fibroid subtypes (F1 and/or F2) of RA. In certain embodiments, a M subtype therapeutic target is selected from one or a combination of genes listed in Table 6 of WO2011/028945. In certain embodiments, a M subtype therapeutic target is selected from one or a combination of genes listed in Table 2 of WO2011/028945. In  
 certain embodiments, a M subtype therapeutic target is selected from one or a combination of  
 25 genes listed in Table 11 of WO2011/028945. In certain embodiments, a M subtype therapeutic target is selected from one or a combination of proteins encoded by one or a combination of genes listed in Table 6 of WO2011/028945. In certain embodiments, a M subtype therapeutic target is selected from one or a combination of proteins encoded by one  
 or a combination of genes listed in Table 2 of WO2011/028945. In certain embodiments, a M  
 30 subtype therapeutic target is selected from one or a combination of proteins encoded by one or a combination of genes listed in Table 11 of WO2011/028945. In certain embodiments, a therapeutic target of M subtype of RA is selected from one or more of CLEC5A, CLEC7A, ALCAM, IL1RAP, IRAK1, NRP2, TREM1, and VEGF.

In another aspect, methods of diagnosing a certain subtype of RA, described herein as the M subtype, comprise measuring the gene expression of one or a combination of genes listed in Table 6 of WO2011/028945, or measuring the amount of protein expressed by one or a combination of genes listed in Table 6 of WO2011/028945. In certain embodiments, one or

5 more of the genes identified in Table 6 of WO2011/028945, or proteins encoded by said genes, are biomarkers of the M subtype. In certain embodiments, methods of diagnosing M subtype RA comprise measuring the gene expression of one or a combination of genes listed in Table 2 of WO2011/028945, or measuring the protein expressed by one or a combination of genes listed in Table 2 of WO2011/028945. In certain embodiments, one or more of the  
10 genes identified in Table 2 of WO2011/028945, or proteins encoded by said genes, are biomarkers of the M subtype. In certain embodiments, methods of diagnosing M subtype RA comprise measuring the gene expression of one or a combination of genes listed in Table 11 of WO2011/028945, or measuring the protein expressed by one or a combination of genes listed in Table 11 of WO2011/028945. In certain embodiments, one or more of the genes  
15 identified in Table 11 of WO2011/028945, or proteins encoded by said genes, are biomarkers of the M subtype. In certain embodiments, methods of diagnosing M subtype of RA comprise measuring the gene expression or protein expression of one or more of ADAM8, CTSB, CXCL3, ICAM1, IL18BP, IL1B, IL8, MMP12, CCL2, VEGFA, and S100A11.

In certain embodiments, a F2 subtype therapeutic target is selected from one or a combination  
20 of genes listed in Table 7 of WO2011/028945. In certain embodiments, a F2 subtype therapeutic target is selected from one or a combination of genes listed in Table 3 of WO2011/028945. In certain embodiments, a F2 subtype therapeutic target is selected from one or a combination of genes listed in Table 12 of WO2011/028945. In certain  
25 embodiments, a F2 subtype therapeutic target is selected from one or a combination of proteins encoded by one or a combination of genes listed in Table 7 of WO2011/028945. In certain embodiments, a F2 subtype therapeutic target is selected from one or a combination of proteins encoded by one or a combination of genes listed in Table 3 of WO2011/028945. In certain embodiments, a F2 subtype therapeutic target is selected from one or a combination of proteins encoded by one or a combination of genes listed in Table 12 of WO2011/028945. In  
30 certain embodiments, a therapeutic target of F2 subtype of RA is selected from one or more of IL17D, IL17RC, TIMP3, and TNFRSF11B.

In another aspect, methods of diagnosing a certain subtype of RA, described herein as the F2 subtype, comprise measuring the gene expression of one or a combination of genes listed in

Table 7 of WO2011/028945, or measuring the protein expressed by one or a combination of genes listed in Table 7 of WO2011/028945. In certain embodiments, one or more of the genes identified in Table 7 of WO2011/028945, or proteins encoded by said genes, are biomarkers of the F2 subtype. In certain embodiments, methods of diagnosing F2 subtype

5 RA comprise measuring the gene expression of one or a combination of genes listed in Table 3 of WO2011/028945, or measuring the protein expressed by one or a combination of genes listed in Table 3. In certain embodiments, one or more of the genes identified in Table 3 of WO2011/028945, or proteins encoded by said genes, are biomarkers of the F2 subtype. In certain embodiments, methods of diagnosing F2 subtype RA comprise measuring the gene  
10 expression of one or a combination of genes listed in Table 12 of WO2011/028945, or measuring the protein expressed by one or a combination of genes listed in Table 12 of WO2011/028945. In certain embodiments, one or more of the genes identified in Table 12 of WO2011/028945, or proteins encoded by said genes, are biomarkers of the F2 subtype. In certain embodiments, methods of diagnosing F2 subtype of RA comprise measuring the gene  
15 expression or protein expression of one or more of FGF10, FGF18, FGF2, LRP6, TGFbeta2, WNT11, BMP6, BTC, CLU, CRLF1, TIMP3, FZD10, FZD7, FZD8, and IL17D.

In certain embodiments, a F1 subtype therapeutic target is selected from one or a combination of genes listed in Table 8 of WO2011/028945. In certain embodiments, a F1 subtype therapeutic target is selected from one or a combination of genes listed in Table 4 of

20 WO2011/028945. In certain embodiments, a F1 subtype therapeutic target is selected from one or a combination of genes listed in Table 13 of WO2011/028945. In certain embodiments, a F1 subtype therapeutic target is selected from one or a combination of proteins encoded by one or a combination of genes listed in Table 8 of WO2011/028945. In certain embodiments, a F1 subtype therapeutic target is selected from one or a combination of  
25 proteins encoded by one or a combination of genes listed in Table 4 of WO2011/028945. In certain embodiments, a F1 subtype therapeutic target is selected from one or a combination of proteins encoded by one or a combination of genes listed in Table 13 of WO2011/028945. In certain embodiments, a therapeutic target of F1 subtype of RA is selected from one or more of CDH11, ITGA11, and CLEC11A.

30 In another aspect, methods of diagnosing a certain subtype of RA, described herein as the F1 subtype, comprise measuring the gene expression of one or a combination of genes listed in Table 8 of WO2011/028945, or measuring the protein expressed by one or a combination of genes listed in Table 8 of WO2011/028945. In certain embodiments, one or more of the



genes identified in Table 8 of WO2011/028945, or proteins encoded by said genes, are biomarkers of the F1 subtype. In certain embodiments, methods of diagnosing F1 subtype RA comprises measuring the gene expression of one or a combination of genes listed in Table 4 of WO2011/028945, or measuring the protein expressed by one or a combination of genes listed in Table 4 of WO2011/028945. In certain embodiments, one or more of the genes identified in Table 4 of WO2011/028945, or proteins encoded by said genes, are biomarkers of the F1 subtype. In certain embodiments, methods of diagnosing F1 subtype RA comprises measuring the gene expression of one or a combination of genes listed in Table 13 of WO2011/028945, or measuring the protein expressed by one or a combination of genes listed in Table 13 of WO2011/028945. In certain embodiments, one or more of the genes identified in Table 13, or proteins encoded by said genes, are biomarkers of the F1 subtype. In certain embodiments, methods of diagnosing F1 subtype of RA comprise measuring the gene expression or protein expression of one or more of ITGA11, MMP11, MMP13, MMP16, MMP28, ADAM12, ADAM22, CTSK, CTHRC1, ENPEP, POSTN, ANGPT2, SFRP2, TIE1, and VWF.

### **Pharmaceutical Formulations**

Pharmaceutical formulations of an anti-IL-34 antibody, a bispecific anti-IL-34/CSF-1 antibody, or an anti-CSF-1R antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming

counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20

hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX<sup>®</sup>, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases. Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958.

Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide an anti-CSF-1 antibody. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules.

The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

### **Therapeutic Methods and Compositions**

Any of the anti-IL-34 antibodies, bispecific anti-IL-34/CSF-1 antibodies or anti CSF-1R antibodies provided herein may be used in therapeutic methods.

In one aspect, an anti-IL-34 antibody or an anti-CSF-1R antibody for use as a medicament is provided. In further aspects, an anti-IL-34 antibody or an anti-CSF-1R antibody for use in

treating myeloid pathogenic immunological diseases is provided. In some embodiments, the myeloid pathogenic immunological disease is rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, lupus nephritis, asthma, osteoporosis, Paget's disease, atherosclerosis, metabolic syndrome, type II diabetes, LSDs (lysosomal storage diseases like but not limited to Cystinosis, Salic acid storage disorder, Gaucher disease), Histiocytosis including but not limited to Rosai-Dorfman disease, Faisalabad histiocytosis, H syndrome, pigmented hypertrichosis with insulin dependent diabetes (PHID). In some embodiments, an anti-IL-34 antibody or an anti-CSF-1R antibody for use in a method of treatment is provided. In some embodiments, the invention provides an anti-IL-34 antibody or an anti-CSF-1R antibody for use in a method of treating an individual having a myeloid pathogenic immunological disease (e.g., rheumatoid arthritis, inflammatory bowel disease or multiple sclerosis) comprising administering to the individual an effective amount of the anti-IL-34 antibody or anti-CSF-1R antibody. In some embodiments, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In some embodiments, the invention provides an anti-IL-34 antibody or an anti-CSF-1R antibody for use in inhibiting binding of IL-34 to CSF-1R. In some embodiments, the invention provides an anti-IL-34 antibody or an anti-CSF-1R antibody for use in a method of inhibiting binding of IL-34 to CSF-1R in an individual comprising administering to the individual an effective amount of the anti-IL-34 antibody or anti-CSF-1R antibody to inhibit binding of IL-34 to CSF-1R. In some embodiments, the invention provides an anti-IL-34 antibody or an anti-CSF-1R antibody for use in neutralizing activity of IL-34. In some embodiments, the invention provides an anti-IL-34 antibody or an anti-CSF-1R antibody for use in a method of neutralizing activity of IL-34 in an individual comprising administering to the individual an effective amount of the anti-IL-34 antibody or anti-CSF-1R antibody to neutralize activity of IL-34. An "individual" according to any of the above embodiments is preferably a human. In a further aspect, the invention provides for the use of an anti-IL-34 antibody or an anti-CSF-1R antibody in the manufacture or preparation of a medicament. In some embodiments, the medicament is for treatment of myeloid pathogenic immunological disease. In some embodiments, the myeloid pathogenic immunological disease is rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, lupus nephritis, asthma, osteoporosis, Paget's disease, atherosclerosis, metabolic syndrome, type II diabetes, LSDs (lysosomal storage diseases like but not limited to Cystinosis, Salic acid

storage disorder, Gaucher disease), Histiocytosis including but not limited to Rosai-Dorfman disease, Faisalabad histiocytosis, H syndrome, pigmented hypertrichosis with insulin dependent diabetes (PHID). In some embodiments, the medicament is for use in a method of treating a myeloid pathogenic immunological disease (e.g., rheumatoid arthritis, inflammatory bowel disease or multiple sclerosis) comprising administering to an individual having a myeloid pathogenic immunological disease (e.g., rheumatoid arthritis, inflammatory bowel disease or multiple sclerosis) an effective amount of the medicament. In some embodiments, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In some embodiments, the medicament is for inhibiting binding of IL-34 to CSF-1R in an individual. In some embodiments, the medicament is for use in a method of inhibiting binding of IL-34 to CSF-1R in an individual comprising administering to the individual an effective amount of the medicament to inhibit binding of IL-34 to CSF-1R in an individual. In some embodiments, the medicament is for neutralizing the activity of IL-34 in an individual. In some embodiments, the medicament is for use in a method of neutralizing the activity of IL-34 in an individual comprising administering to the individual an effective amount of the medicament to neutralize the activity of IL-34 in an individual. An “individual” according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for treating a myeloid pathogenic immunological disease. In some embodiments, the myeloid pathogenic immunological disease is rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, lupus nephritis, asthma, osteoporosis, Paget’s disease, atherosclerosis, metabolic syndrome, type II diabetes, LSDs (lysosomal storage diseases like but not limited to Cytostinosis, Salic acid storage disorder, Gaucher disease), Histiocytosis including but not limited to Rosai-Dorfman disease, Faisalabad histiocytosis, H syndrome, pigmented hypertrichosis with insulin dependent diabetes (PHID). In some embodiments, the method comprises administering to an individual having a myeloid pathogenic immunological disease (e.g., rheumatoid arthritis, inflammatory bowel disease or multiple sclerosis) an effective amount of the anti-IL-34 antibody or anti-CSF-1R antibody. In some embodiments, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An “individual” according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for inhibiting binding of IL-34 to CSF-1R in an individual. In some embodiments, the method comprises administering to the individual an effective amount of an anti-IL-34 antibody or an anti CSF-1R antibody to inhibit binding of IL-34 to CSF-1R in an individual. In a further aspect, the invention provides a method for neutralizing activity of IL-34 in an individual. In some embodiments, the method comprises administering to the individual an effective amount of an anti-IL-34 antibody or an anti CSF-1R antibody to neutralize activity of IL-34 in an individual. In some embodiments, an "individual" is a human.

In one aspect, a bispecific anti-IL-34/CSF-1 antibody for use as a medicament is provided. In further aspects, a bispecific anti-IL-34/CSF-1 antibody for use in treating myeloid pathogenic immunological disease is provided. In some embodiments, the myeloid pathogenic immunological disease is rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, lupus nephritis, asthma, osteoporosis, Paget's disease, atherosclerosis, metabolic syndrome, type II diabetes, LSDs (lysosomal storage diseases like but not limited to Cystinosis, Sialic acid storage disorder, Gaucher disease), Histiocytosis including but not limited to Rosai-Dorfman disease, Faisalabad histiocytosis, H syndrome, pigmented hypertrichosis with insulin dependent diabetes (PHID). In some embodiments, a bispecific anti-IL-34/CSF-1 antibody for use in a method of treatment is provided. In some embodiments, the invention provides a bispecific anti-IL-34/CSF-1 antibody for use in a method of treating an individual having myeloid pathogenic immunological disease (e.g., rheumatoid arthritis, inflammatory bowel disease or multiple sclerosis) comprising administering to the individual an effective amount of the bispecific anti-IL-34/CSF-1 antibody. In some embodiments, the invention provides a bispecific anti-IL-34/CSF-1 antibody for use in inhibiting binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-1R. In some embodiments, the invention provides a bispecific anti-IL-34/CSF-1 antibody for use in a method of inhibiting binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-1R in an individual comprising administering to the individual an effective amount of the bispecific anti-IL-34/CSF-1 antibody to inhibit binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-1R. In some embodiments, the invention provides a bispecific anti-IL-34/CSF-1 antibody for use in neutralizing activity of IL-34 and/or CSF-1. In some embodiments, the invention provides a bispecific anti-IL-34/CSF-1 antibody for use in a method of neutralizing activity of IL-34 and/or CSF-1 in an individual comprising administering to the individual an effective amount of the bispecific anti-IL-34/CSF-1

antibody to neutralize activity of IL-34 and/or CSF-1. An “individual” according to any of the above embodiments is preferably a human.

In a further aspect, the invention provides for the use of a bispecific anti-IL-34/CSF-1

antibody in the manufacture or preparation of a medicament. In some embodiments, the

5 medicament is for treatment of myeloid pathogenic immunological disease. In some  
embodiments, the myeloid pathogenic immunological disease is rheumatoid arthritis,  
inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, lupus  
nephritis, asthma, osteoporosis, Paget’s disease, atherosclerosis, metabolic syndrome, type II  
diabetes, LSDs (lysosomal storage diseases like but not limited to Cystinosis, Salic acid  
10 storage disorder, Gaucher disease), Histiocytosis including but not limited to Rosai-Dorfman  
disease, Faisalabad histiocytosis, H syndrome, pigmented hypertrichosis with insulin  
dependent diabetes (PHID). In some embodiments, the medicament is for use in a method of  
treating myeloid pathogenic immunological disease (e.g., rheumatoid arthritis, inflammatory  
bowel disease or multiple sclerosis) comprising administering to an individual having  
15 myeloid pathogenic immunological disease (e.g., rheumatoid arthritis, inflammatory bowel  
disease or multiple sclerosis) an effective amount of the medicament. In some embodiments,  
the medicament is for inhibiting binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-  
1R in an individual. In some embodiments, the medicament is for use in a method of  
inhibiting binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-1R in an individual  
20 comprising administering to the individual an effective amount of the medicament to inhibit  
binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-1R in an individual. In some  
embodiments, the medicament is for neutralizing the activity of IL-34 and/or CSF-1 in an  
individual. In some embodiments, the medicament is for use in a method of neutralizing the  
activity of IL-34 and/or CSF-1 in an individual comprising administering to the individual an  
25 effective amount of the medicament to neutralize the activity of IL-34 and/or CSF-1 in an  
individual. An “individual” according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for treating a myeloid pathogenic

immunological disease. In some embodiments, the myeloid pathogenic immunological

disease is rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, systemic lupus

30 erythematosus, lupus nephritis, asthma, osteoporosis, Paget’s disease, atherosclerosis,  
metabolic syndrome, type II diabetes, LSDs (lysosomal storage diseases like but not limited  
to Cystinosis, Salic acid storage disorder, Gaucher disease), Histiocytosis including but not  
limited to Rosai-Dorfman disease, Faisalabad histiocytosis, H syndrome, pigmented

hypertrichosis with insulin dependent diabetes (PHID). In some embodiments, the method comprises administering to an individual having a myeloid pathogenic immunological disease (e.g., rheumatoid arthritis, inflammatory bowel disease or multiple sclerosis) an effective amount of the a bispecific anti-IL-34/CSF-1 antibody. An “individual” according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for inhibiting binding of IL-34 to CSF-1 and binding of CSF-1 to CSF-1R in an individual. In some embodiments, the method comprises administering to the individual an effective amount of a bispecific anti-IL-34/CSF-1 antibody to inhibit binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-1R in an individual. In a further aspect, the invention provides a method for neutralizing activity of IL-34 and/or CSF-1 in an individual. In some embodiments, the method comprises administering to the individual an effective amount of a bispecific anti-IL-34/CSF-1 antibody to neutralize activity of IL-34 and/or IL-34 in an individual. In some embodiments, an “individual” is a human.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-IL-34 antibodies, bispecific anti-IL-34/CSF-1 antibodies, or anti-CSF-1R antibodies provided herein, e.g., for use in any of the above therapeutic methods. In some embodiments, a pharmaceutical formulation comprises any of the anti-IL-34 antibodies, bispecific anti-IL-34/CSF-1 antibodies, or anti-CSF-1R antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-IL-34 antibodies, bispecific anti-IL-34/CSF-1 antibodies, or anti-CSF-1R antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In some embodiments, an additional therapeutic agent is an anti-CSF1-antibody.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant.

In further aspects, an anti-IL-34 antibody and an anti-CSF-1 antibody for use in treating myeloid pathogenic immunological disease are provided. In some embodiments, the myeloid

pathogenic immunological disease is rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, lupus nephritis, asthma, osteoporosis, Paget's disease, atherosclerosis, metabolic syndrome, type II diabetes, LSDs (lysosomal storage diseases like but not limited to Cystinosis, Salic acid storage disorder, Gaucher disease), Histiocytosis including but not limited to Rosai-Dorfman disease, Faisalabad histiocytosis, H syndrome, pigmented hypertrichosis with insulin dependent diabetes (PHID). In some embodiments, an anti-IL-34 antibody and an anti-CSF-1 antibody for use in a method of treatment are provided. In some embodiments, the invention provides an anti-IL-34 antibody and an anti-CSF-1 antibody for use in a method of treating an individual having myeloid pathogenic immunological disease (e.g., rheumatoid arthritis, inflammatory bowel disease or multiple sclerosis) comprising administering to the individual an effective amount of the anti-IL-34 antibody in conjunction with an anti-CSF-1 antibody. In some embodiments, the invention provides an anti-IL-34 antibody and an anti-CSF-1 antibody for use in inhibiting binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-1R. In some embodiments, the invention provides an anti-IL-34 antibody and an anti-CSF-1 antibody for use in a method of inhibiting binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-1R in an individual comprising administering to the individual an effective amount of the anti-IL-34 antibody in conjunction with an anti-CSF-1 antibody to inhibit binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-1R. In some embodiments, the invention provides an anti-IL-34 antibody and an anti-CSF-1 antibody for use in neutralizing activity of IL-34 and/or CSF-1. In some embodiments, the invention provides an anti-IL-34 antibody and an anti-CSF-1 antibody for use in a method of neutralizing activity of IL-34 and/or CSF-1 in an individual comprising administering to the individual an effective amount of the anti-IL-34 antibody in conjunction with an anti-CSF-1 antibody to neutralize activity of IL-34 and/or CSF-1. An "individual" according to any of the above embodiments is preferably a human.

In a further aspect, the invention provides a method for treating a myeloid pathogenic immunological disease. In some embodiments, the myeloid pathogenic immunological disease is rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, lupus nephritis, asthma, osteoporosis, Paget's disease, atherosclerosis, metabolic syndrome, type II diabetes, LSDs (lysosomal storage diseases like but not limited to Cystinosis, Salic acid storage disorder, Gaucher disease), Histiocytosis including but not limited to Rosai-Dorfman disease, Faisalabad histiocytosis, H syndrome, pigmented hypertrichosis with insulin dependent diabetes (PHID). In some embodiments, the method



comprises administering to an individual having a myeloid pathogenic immunological disease (e.g., rheumatoid arthritis, inflammatory bowel disease or multiple sclerosis) an effective amount of an anti-IL-34 antibody in conjunction with an anti-CSF-1 antibody. An “individual” according to any of the above embodiments may be a human.

- 5 In a further aspect, the invention provides a method for inhibiting binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-1R in an individual. In some embodiments, the method comprises administering to the individual an effective amount of an anti-IL-34 antibody in conjunction with an anti CSF-1 antibody to inhibit binding of IL-34 to CSF-1R and binding of CSF-1 to CSF-1R in an individual. In some embodiments, an “individual” is a human.
- 10 In a further aspect, the invention provides a method for neutralizing activity of IL-34 and/or CSF-1 in an individual. In some embodiments, the method comprises administering to the individual an effective amount of an anti-IL-34 antibody in conjunction with an anti CSF-1 antibody to neutralize activity of IL-34 and/or CSF-1 in an individual. In some embodiments, an “individual” is a human.
- 15 The anti-IL-34 antibody, anti-CSF-1R antibody, and bispecific anti-IL-34/CSF-1 antibody described herein may be used for targeting the myeloid stroma of a tumor. In some embodiments, the antibody is used for treating cancer (such as colon, lung, breast, prostate and uterine cancer, etc.). In some embodiments, the antibody is administered to an individual in conjunction with another anti-cancer therapy for treating the cancer in the individual. In
- 20 some embodiments, the anti-cancer therapy is a treatment with Herceptin<sup>®</sup>, Avastin<sup>®</sup>, or Tarceva<sup>®</sup>.

The anti-IL-34 antibody, anti-CSF-1R antibody, and bispecific anti-IL-34/CSF-1 antibody described herein may be also used for treating lysosomal storage disease (LSD), and autoimmune disease including but is not limited to rheumatoid arthritis, inflammatory bowel

25 disease (IBD, e.g., Crohn’s, ulcerative colitis), multiple sclerosis, vascular diseases including but not limited to atherosclerosis, myocardial infarction and angina, osteoporosis, Alzheimer’s disease, diabetes mellitus (Type 1 and/or Type 2), infectious diseases, and cancer.

Lysosomal Storage Disease (LSD) is a metabolic disorder that results from defects in lysosomal function. Lysosomal storage diseases result when a specific organelle in the body's

30 cells – the lysosome – malfunctions. Examples of LSDs include, but are not limited to, diseases caused by a protein that is deficient/defective such as, defective/deficient lysosomal hydrolases (e.g., sphingolipidoses like gangliosidosis, Gaucher and various Niemann-Pick diseases), posttranslationally modified sulfatases (e.g., Multiple sulfatase deficiency),

- defective/deficient membrane transport proteins such as nucleotide/nucleoside transporters or N-acetylglucosamine-1-phosphate transferase (e.g., mucopolipidosis type II and IIIA), defective/deficient enzyme protecting proteins such as cathepsin A (e.g., GM2-AP deficiency, variant AB, Niemann-Pick disease, type C2, SAP deficiency), defective/deficient
- 5 transmembrane proteins such as M-CSFR, ENT3, NPC1 and sialin (e.g., Niemann-Pick disease, type C1, Salla disease). Categories of LSDs include lipid storage disorders (e.g., sphingolipidoses), gangliosidosis (e.g., Tay-Sachs disease), leukodystrophies, mucopolysaccharidoses (including Hunter syndrome and Hurler disease), glycoprotein storage disorders (e.g., Pompe disease) and mucolipidoses.
- 10 More common LSDs are known as the following: Activator Deficiency/GM2 Gangliosidosis, Alpha-mannosidosis, Aspartylglucosaminuria, Cholesteryl ester storage disease, Chronic Hexosaminidase A Deficiency, Cystinosis, Danon disease, Fabry disease, Farber disease, Fucosidosis, Galactosialidosis, Gaucher Disease (Type I, Type II, Type III), GM1 gangliosidosis, (Infantile, Late infantile/Juvenile , Adult/Chronic), I-Cell
- 15 disease/Mucolipidosis II, Infantile Free Sialic Acid Storage Disease/ISSD, Juvenile Hexosaminidase A Deficiency, Krabbe disease (Infantile Onset, Late Onset), Metachromatic Leukodystrophy, Mucopolysaccharidoses disorders (e.g., Pseudo-Hurler polydystrophy/Mucolipidosis IIIA, MPSI Hurler Syndrome, MPSI Scheie Syndrome, MPS I Hurler-Scheie Syndrome, MPS II Hunter syndrome, Sanfilippo syndrome Type A/MPS III A, Sanfilippo syndrome Type B/MPS III B, Sanfilippo syndrome Type C/MPS III C , Sanfilippo syndrome Type D/MPS III D, Morquio Type A/MPS IVA, Morquio Type B/MPS IVB, MPS
- 20 IX Hyaluronidase Deficiency, MPS VI Maroteaux-Lamy, MPS VII Sly Syndrome, Mucolipidosis I/Sialidosis, Mucolipidosis IIIC, Mucolipidosis type IV), Multiple sulfatase deficiency, Niemann-Pick Disease (Type A, Type B, Type C), Neuronal Ceroid
- 25 Lipofuscinoses (e.g., CLN6 disease - Atypical Late Infantile, Late Onset variant, Early Juvenile, Batten-Spielmeyer-Vogt/Juvenile NCL/CLN3 disease, Finnish Variant Late Infantile CLN5, Jansky-Bielschowsky disease/Late infantile CLN2/TPP1 Disease, Kufs/Adult-onset NCL/CLN4 disease, Northern Epilepsy/variant late infantile CLN8, Santavuori-Haltia/Infantile CLN1/PPT disease, Beta-mannosidosis), Pompe disease/Glycogen
- 30 storage disease type II, Pycnodysostosis, Sandhoff disease/Adult Onset/GM2 Gangliosidosis. Sandhoff disease/GM2 gangliosidosis – Infantile, Sandhoff disease/GM2 gangliosidosis – Juvenile, Schindler disease, Salla disease/Sialic Acid Storage Disease, Tay-Sachs/GM2 gangliosidosis, and Wolman disease.

An “autoimmune disease” described herein is a disease or disorder arising from and directed against an individual’s own tissues or a co-segregate or manifestation thereof or resulting condition therefrom. Examples of autoimmune diseases or disorders include, but are not limited to arthritis (rheumatoid arthritis such as acute arthritis, chronic rheumatoid arthritis, 5 gouty arthritis, acute gouty arthritis, chronic inflammatory arthritis, degenerative arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, vertebral arthritis, and juvenile-onset rheumatoid arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, and ankylosing spondylitis), inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, guttate 10 psoriasis, pustular psoriasis, and psoriasis of the nails, dermatitis including contact dermatitis, chronic contact dermatitis, allergic dermatitis, allergic contact dermatitis, dermatitis herpetiformis, and atopic dermatitis, x-linked hyper IgM syndrome, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, 15 scleroderma (including systemic scleroderma), sclerosis such as systemic sclerosis, multiple sclerosis (MS) such as spino-optical MS, primary progressive MS (PPMS), and relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, and ataxic sclerosis, inflammatory bowel disease (IBD) (for example, Crohn's disease, autoimmune-mediated gastrointestinal diseases, colitis such as ulcerative 20 colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis polyposa, necrotizing enterocolitis, and transmural colitis, and autoimmune inflammatory bowel disease), pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, episcleritis), respiratory distress syndrome, including adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological 25 disorder, rheumatoid spondylitis, sudden hearing loss, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis such as Rasmussen's encephalitis and limbic and/or brainstem encephalitis, uveitis, such as anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior uveitis, or autoimmune uveitis, glomerulonephritis (GN) with and without nephrotic syndrome such as 30 chronic or acute glomerulonephritis such as primary GN, immune-mediated GN, membranous GN (membranous nephropathy), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous proliferative GN (MPGN), including Type I and Type II, and rapidly progressive GN, allergic conditions, allergic reaction, eczema

including allergic or atopic eczema, asthma such as asthma bronchiale, bronchial asthma, and auto-immune asthma, conditions involving infiltration of T cells and chronic inflammatory responses, chronic pulmonary inflammatory disease, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) or systemic lupus erythematoses such as cutaneous SLE, subacute cutaneous lupus erythematosus, neonatal lupus syndrome (NLE), lupus erythematosus disseminatus, lupus (including nephritis, cerebritis, pediatric, non-renal, extra-renal, discoid, alopecia), juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), adult onset diabetes mellitus (Type II diabetes), autoimmune diabetes, idiopathic diabetes insipidus, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitides, including vasculitis (including large vessel vasculitis (including polymyalgia rheumatica and giant cell arteritis or Takayasu's arteritis), medium vessel vasculitis (including Kawasaki's disease and polyarteritis nodosa), microscopic polyarteritis, CNS vasculitis, necrotizing, cutaneous, or hypersensitivity vasculitis, systemic necrotizing vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS)), temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia (anemia perniciosa), Addison's disease, pure red cell anemia or aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex-mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet's or Behcet's disease, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous and skin pemphigoid, pemphigus (including pemphigus vulgaris, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, and pemphigus erythematosus), autoimmune polyendocrinopathies, Reiter's disease or syndrome, immune complex nephritis, antibody-mediated nephritis, neuromyelitis optica, polyneuropathies, chronic neuropathy such as IgM polyneuropathies or IgM-mediated neuropathy, thrombocytopenia (as developed by myocardial infarction patients, for example), including thrombotic thrombocytopenic purpura (TTP) and autoimmune or immune-mediated

thrombocytopenia such as idiopathic thrombocytopenic purpura (ITP) including chronic or acute ITP, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune endocrine diseases including thyroiditis such as autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis (Hashimoto's thyroiditis), or subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis such as allergic encephalomyelitis or encephalomyelitis allergica and experimental allergic encephalomyelitis (EAE), myasthenia gravis such as thymoma-associated myasthenia gravis, cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, giant cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis, bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barré syndrome, Berger's disease (IgA nephropathy), idiopathic IgA nephropathy, linear IgA dermatosis, primary biliary cirrhosis, pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac disease, Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia, amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AIED), autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory or relapsed polychondritis, pulmonary alveolar proteinosis, amyloidosis, scleritis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis (e.g., benign monoclonal gammopathy and monoclonal gammopathy of undetermined significance, MGUS), peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal segmental glomerulosclerosis (FSGS), endocrine ophthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases, diabetic nephropathy, Dressler's syndrome, alopecia areata, CREST syndrome (calcinosis, Raynaud's phenomenon,

esophageal dysmotility, sclerodactyly, and telangiectasia), male and female autoimmune infertility, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciiitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis, or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, echovirus infection, cardiomyopathy, Alzheimer's disease, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis obliterans, thyrotoxicosis, tabes dorsalis, chorioiditis, giant cell polymyalgia, endocrine ophthalmopathy, chronic hypersensitivity pneumonitis, keratoconjunctivitis sicca, epidemic keratoconjunctivitis, idiopathic nephritic syndrome, minimal change nephropathy, benign familial and ischemia-reperfusion injury, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders, aspermiogenesis, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmia phacoanaphylactica, enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensorineural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, leucopenia, mononucleosis infectiosa, transverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia sympathica, orchitis granulomatosa, pancreatitis, polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyroiditis, acquired spenic atrophy, infertility due to antispermatozoan antibodies, non-malignant thymoma, vitiligo, SCID and Epstein-Barr virus-associated diseases, acquired immune deficiency syndrome (AIDS), parasitic diseases such as Leishmania, toxic-shock syndrome, food poisoning, conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving

leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease, allergic neuritis, autoimmune polyendocrinopathies, oophoritis, primary myxedema, autoimmune atrophic gastritis, sympathetic ophthalmia, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulinitis, polyendocrine failure, peripheral neuropathy, autoimmune polyglandular syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), alopecia totalis, dilated cardiomyopathy, epidermolysis bullosa acquisita (EBA), hemochromatosis, myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent sinusitis, acute or chronic sinusitis, ethmoid, frontal, maxillary, or sphenoid sinusitis, an eosinophil-related disorder such as eosinophilia, pulmonary infiltration eosinophilia, eosinophilia-myalgia syndrome, Löffler's syndrome, chronic eosinophilic pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma, or granulomas containing eosinophils, anaphylaxis, seronegative spondyloarthritides, polyendocrine autoimmune disease, sclerosing cholangitis, sclera, episclera, chronic mucocutaneous candidiasis, Bruton's syndrome, transient hypogammaglobulinemia of infancy, Wiskott-Aldrich syndrome, ataxia telangiectasia, autoimmune disorders associated with collagen disease, rheumatism, neurological disease, ischemic re-perfusion disorder, reduction in blood pressure response, vascular dysfunction, angiectasis, tissue injury, cardiovascular ischemia, hyperalgesia, cerebral ischemia, and disease accompanying vascularization, allergic hypersensitivity disorders, glomerulonephritides, reperfusion injury, reperfusion injury of myocardial or other tissues, dermatoses with acute inflammatory components, acute purulent meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, acute serious inflammation, chronic intractable inflammation, pyelitis, pneumonocirrhosis, diabetic retinopathy, diabetic large-artery disorder, endarterial hyperplasia, peptic ulcer, valvulitis, and endometriosis.

An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g., by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules

including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g., 0.1mg/kg-10mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g., every week or every three weeks (e.g., such that the patient receives from about two to about twenty, or e.g., about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. An



exemplary dosing regimen comprises administering. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

#### **Articles of Manufacture or Kit**

5 In another aspect of the invention, an article of manufacture or kit containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials  
10 such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert  
15 indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of  
20 the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. In some embodiments, the article of manufacture comprises an anti-IL-34 antibody as described herein. In some embodiments, the article of manufacture comprises a bispecific IL-34/CSF-1 antibody as described herein. In some embodiments, the article of manufacture comprises an anti-CSF-1R antibody as described  
25 herein. In some embodiments, the article of manufacture comprises an anti-I-34 as described herein and an anti-CSF-1 antibody. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from  
30 a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

## EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

### 5           **Example 1: Structures of IL-34, IL-34/CSF-1R complexes and IL-34/antibody complexes**

#### ***Methods***

##### *Protein Expression and Purification*

The coding sequences of human IL-34 active core (residues N21–V193 for hIL-34s and  
10 residues N21-P242 for hIL-34fl) fused with a C-terminal Flag-tag or His6-tag, and the extracellular domains of human CSF-1R (residues 20–299 for domains D1–D3 and residues 20–512 for domains D1–D5) attached with a C-terminal His6-tag, were cloned into the pAcGP67 vector (BD Biosciences). Recombinant baculovirus was generated by co-transfecting sf9 cells with the pAcGP67A constructs and linearized baculovirus DNA in ESF  
15 921 media (Expression Systems LLC, Woodland, CA) using the BaculoGold™ Expression System according to manufacturer's instructions (Pharmingen). Virus was generated through three rounds of amplification and 4 ml of the round-3 stock was used to infect 1 liter of *Tni.PRO* cells at a density of  $2 \times 10^6$  cells/ml. Cells were grown 48 h at 27 °C and removed from the media by centrifugation. Supernatant was removed from cells and supplemented  
20 with 1 mM NiCl<sub>2</sub>, 5 mM CaCl<sub>2</sub>, in 50 mM Tris-HCl pH 7.5. The proteins in the supernatant were captured by Ni-NTA column (Qiagen) using gravity flow, washed with 30 ml wash buffer (50 mM Tris-HCl pH 7.5, 300 mM NaCl, 10 mM imidazole), and eluted off the column with elution buffer (50 mM Tris-HCl pH 7.5, 300 mM NaCl, 300 mM imidazole). Protein was concentrated and was further purified over a size exclusion column (HiLoad  
25 16/60 Superdex 200, GE Biosciences) equilibrated with 5 mM Tris-HCl pH 7.5 and 100 mM NaCl. Fractions containing proteins of interest were analyzed by SDS-PAGE, pooled, and concentration was determined by the absorbance at 280 nm.

##### *Analytical Gel Filtration*

500 µl protein samples at 1 mg/mL concentration were injected sequentially into a Superdex  
30 200 10/300 GL column (GE Biosciences) equilibrated with PBS. The same column was calibrated by a mixture of protein molecular weight standards (Bio-Rad), and the elution

volumes of the proteins of interest were used to estimate their corresponding molecular weight.

#### *Isothermal Titration Calorimetry*

All protein samples were buffer-exchanged into PBS buffer using a Superdex 200 10/300 GL column prior to ITC experiments to minimize buffer dilution effects. Calorimetric titrations were carried out on a VP-ITC 200 calorimeter (MicroCal, Northampton, MA), and the data were processed with MicroCal Origin 7.0 software. The IL-34 or CSF-1 protein, the injectant, was added to CSF-1R D1-D3, or CSF-1R D1-D5, over the course of a number of injections until the receptors were fully saturated. The CSF-1R D1-D3 or D-D5, the injectant, was added to cells containing IL-34, CSF-1, or IL-34/CSF-1R D1-D3 complex over the course of a number of injections until the receptors were fully saturated.

#### *Affinity measurements using Bio-Layer Interferometry (BLI)*

##### IL-34 or CSF-1 binding to CSF-1RD1-D3

Binding assays were conducted using an Octet RED384<sup>TM</sup> BLI instrument (fortéBio).

Biotinylated IL-34 or CSF-1 were immobilized on streptavidin-coated biosensor, washed, and transferred into reaction buffer (1x Kinetics assay buffer, fortéBio #18-5032) containing four-fold serial dilutions of CSF-1RD1-D3 (1600 nM to 25 nM for IL-34 and 3200 nM to 50 nM for CSF-1). Association reactions were monitored until the binding reached steady-state. Subsequently, the bound materials were transferred into reaction buffer and dissociation reactions were monitored to ensure reversible binding between cytokines and CSF-1RD1-D3. Association rates ( $k_{on}$ ) and dissociation rates ( $k_{off}$ ) were calculated using a simple single-site binding equation. The equilibrium dissociation constant ( $K_d$ ) was calculated as the ratio  $k_{off} / k_{on}$ . Binding kinetics was measured at 30°C and samples were agitated @ 1000 rpm.

Antibody affinities were also evaluated using BIACORE 3000. The affinity was generally in line with the BLI measurement. Anti-IL-34 human IgGs were captured by mouse anti-human IgG coated on the CM5 sensor chip to achieve approximately 250 response units (RU). For kinetics measurements, two-fold serial dilutions of human and mouse IL-34 (3.9nM to 500nM) were injected in PBT buffer (PBS with 0.05% Tween 20) at 25°C with a flow rate of 30ml/min. Association rates ( $k_{on}$ ) and dissociation rates ( $k_{off}$ ) were calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2). The equilibrium dissociation constant ( $K_D$ ) was calculated as the ratio  $k_{off}/k_{on}$ .

## Crytallization and Structure Determination

### *hIL-34s\_CFlag/YW405.3 Fab*

Equal molar ratios of hIL-34s\_CFlag and YW405.3 Fab were mixed and subjected to a final gel filtration step using HiLoad 16/60 Superdex 200 column, and concentrated to 30 mg/ml before crystallization trials. Orthorhombic crystals of the hIL-34s\_CFlag/YW405.3 Fab complex were grown at 19 °C from Hampton Research Index HT H02 (86) containing 0.2 M Potassium sodium tartrate tetrahydrate, 20% w/v Polyethylene glycol 3,350.

### *hIL-34s\_CFlag*

Two dimensional plates of hIL-34s\_CFlag were obtained at 19 °C by mixing equal volumes of the protein at 10 mg/ml and reservoir solution containing 0.1 M Hepes pH 7.5, 10% w/v Polyethylene glycol 6,000, 5% v/v (+/-)-2-Methyl-2,4-pentanediol (Hampton Research Crystal Screen HT G06 (78)).

### *hIL-34s\_CHis/hCSF-1R D1-D3 CHis*

hIL-34s\_CHis and hCSF-1R D1-D3\_CHis were co-expressed in insect cells and purified by nickel-affinity and size exclusion chromatography using HiLoad 16/60 Superdex 200 column. The complex was concentrated to 20 mg/ml and screened with the sitting-drop vapor-diffusion technique. Spindle-shaped crystals of hIL-34s\_CHis/hCSF-1R D1-D3 CHis complex grew at 19 °C from Qiagen Protein Complex Screen A02 (2) containing 0.1M CaAcet, 0.1M Mes pH 6, 15% PEG 400.

### *hIL-34s\_CFlag/YW404.33.56 Fab*

The Fab fragment of YW404.33.56 was mixed with hIL-34s\_CFlag at a 1:1 molar ratio and further purified by a HiLoad 16/60 Superdex 200 column and concentrated to 20 mg/ml. A single hIL-34s\_CFlag/YW404.33.56 Fab complex crystal grew after one month at 19 °C over a reservoir solution of 0.2 M Ammonium phosphate monobasic, 0.1 M Tris pH 8.5, 50% v/v (+/-)-2-Methyl-2,4-pentanediol.

## *Data Collection*

All cryostabilized crystals were flash frozen in liquid nitrogen prior to data collection. All datasets were collected at ALS BL 5.0.2, using ADSC Q315 CCD detectors. The diffraction images were indexed, integrated and scaled using HKL2000 (Otwinowski et al., *Methods in*

*Enzymology* **276**: 307-326 (1997)). A summary of all statistics for data collection, phasing, and crystallographic refinement is given in Table 2.

Table 2. X-ray data collection, phasing and refinement statistics

Data collection	hIL-34s	hIL-34s/hCSF-1R	hIL-34s/YW405.3 Fab	hIL-34s/YW404.33.56 Fab
Wavelength (Å)				
Space group	P2 <sub>1</sub>	P3 <sub>1</sub> 21	C2	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Cell dimension	a=57.694Å b=77.866Å c=58.083Å α=γ=90° β=103.245°	a=b=101.361Å c=175.252Å α=β=90° γ=120.000°	a=117.917Å b=181.822Å c=79.254Å α=γ=90° β=118.268°	a=105.549Å b=122.465Å c=149.155Å α=β=γ=90°
Resolution (Å)	50.0-1.85 (1.92-1.85)	50.0-3.0 (3.11-3.00)	50.0-2.6 (2.69-2.60)	50.0-3.0 (3.11-3.00)
Total observations	154925	164357	243871	208888
Unique observations	42509	21502	44009	38972
I/SigI	11.3 (2.0)	23.5 (2.8)	11.0 (2.3)	16.7 (2.2)
Redundancy	3.6 (3.2)	7.6 (7.0)	5.5 (5.4)	5.4 (5.0)
Completeness (%)	99.8 (99.0)	99.8 (99.8)	99.3 (96.9)	99.6 (99.9)
R <sub>sym</sub> <sup>a</sup> (%)	9.3 (51.9)	6.1 (62.7)	11.1 (60.9)	8.6 (62.3)
Refinement				
Resolution (Å)	50.0-1.85	50.0-3.0	50.0-2.6	50.0-3.0
No. of protein atoms	2614	4724	8729	8948
No. of sugar atoms	78	134	122	111
No of waters	486	16	126	50
R <sub>work</sub>	17.4%	28.4%	22.8%	23.5%
R <sub>free</sub> <sup>b</sup>	20.3%	31.3%	25.9%	27.3%
rmsd bond lengths	0.015	0.004	0.006	0.006
rmsd bond angles	1.402	0.881	0.979	0.927
Ramachandran plot				
Most favored (%)	93.9	88.2	89.6	90.5
Additional allowed (%)	6.1	10.6	9.9	8.8
Generously allowed (%)	0.0	0.4	0.1	0.7
Disallowed (%)	0.0	0.8	0.4	0.0

5 Values in parentheses represent the highest resolution shell

$aR_{\text{sym}}(I) = \sum hkl \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum hkl \sum_i I_i(hkl)$  where the summations are over *i* observations of each reflections and all *hkl*.  $\langle I(hkl) \rangle$  is the average intensity of the *i* observations.  $R_{\text{work}} = |F(\text{obs}) - F(\text{calc})|/F(\text{obs})$

$bR^{\text{free}}$  is calculated for 5% of randomly selected reflections not used in the refinement

*Structure Determination and Refinement*

Complete datasets to 1.85 Å, 3.0 Å, 2.6 Å, and 3.0 Å were collected from single crystals of hIL-34s, hIL-34s/CSF-1R D1-D3 complex, hIL-34s/YW405.3 Fab complex, and hIL-34s/YW404.33.56 Fab complex, respectively (Table 2).

5 *hIL-34s\_CFlag/YW405.3 Fab and hIL-34s\_CFlag*

The hIL-34s\_CFlag/YW405.3 Fab complex structure was solved by molecular replacement using the program PHASER (Storoni et al., *Acta crystallographica Section D, Biological crystallography* **60**: 432-438 (2004)) with search models generated from a single Fab (Protein Data Bank (PDB) accession number 2QQN) by searching for the Fv and Fc region of the Fab  
10 sequentially. A single unambiguous solution including 2 Fabs was found revealing very limited electron density for IL-34 outside the CDR regions of the Fab. The phases from this initial solution were drastically improved by imposing solvent flattening, 2-fold NCS averaging using prime-and-switch map plot routine in PHENIX AutoBuild (Adams et al., *Acta crystallographica Section D, Biological crystallography* **66**: 213-221 (2010)). The  
15 resulting map was of sufficient high quality to allow manual tracing of the entire backbone of IL-34 in Coot. This partial IL-34 model was fed into the 1.85 Å resolution dataset of hIL-34s\_CFlag by molecular replacement and subject to model rebuilding using Coot (Emsley et al., *Acta crystallographica Section D, Biological crystallography* **66**: 486-501 (2010)), and refinement using Refmac (Murshudov et al., *Acta crystallographica Section D, Biological  
20 crystallography* **53**: 240-255 (1997)) iteratively. In the final rounds of refinement 4 N-acetylglucosamine groups and 4 mannose moieties were added to residues Asn75 of IL-34 and 486 water molecules were included in the final model. The completed IL-34 model was then used for rebuilding and refining the hIL-34s\_CFlag/YW405.3 Fab complex structure to convergence at 2.6 Å resolution.

25 *hIL-34s\_CHis/hCSF-1R D1-D3 CHis*

The individual components of the hIL-34s\_CHis/hCSF-1R D1-D3 CHis complex were sequentially located using the molecular replacement program PHASER, with hIL-34s, murine CSF-1R D1-D2 and murine CSF-1R D3 (PDB ID 3EJJ) as the search models. CSF-1R D1-D3 was rebuilt and refined according to the human amino acid sequence and the final  
30 hIL-34s\_CHis/hCSF-1R D1-D3 CHis model was completed by interactive refinement in Refmac and model-building in Coot.

*hIL-34s\_CFlag/YW404.33.56 Fab*

The hIL-34s\_CFlag in complex with the blocking Fab YW405.33.56 was determined by the molecular replacement method implemented in the program PHASER with the structure of hIL-34s and the Fv and Fc region of a Fab with similar framework, followed by manual fitting of the CDR regions of the YW404.33.56 Fab. Several rounds of model rebuilding and structure refinement were carried out using the 3.0 Å dataset until convergence was reached. A summary of the refinement statistics and the stereochemistry analysis of all four structures are given in Table 2. The program MolProbity (Chen et al., *Acta crystallographica Section D, Biological crystallography* **66**: 12-21 (2010)) was used to inspect the quality of the final models. Coordinates have been deposited with the Protein Data Bank (www.rcsb.org) with accession codes xxx, yyy, zzz, www for hIL-34s, hIL-34s/hCSF-1R D1-D3, hIL-34s/YW404.33.56 Fab and hIL-34s/YW405.3, respectively. Figures were prepared using the program PyMol (WorldWideWeb at pymol.sourceforge.net)

*Human mononuclear cells and the cell viability / proliferation assay*

Human peripheral blood mononuclear cells (PBMC) were isolated by gradient centrifugation over Ficoll. In the cell viability / proliferation assay,  $1 \times 10^4$  freshly isolated PBMC per well in 96-well plate were stimulated with IL-34 (full length and short version) or CSF-1 in serial dilutions. After incubation at 37 °C for 72 hours, ATP levels in cells were measured by CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega, cat. #G7571) for determining cell viability / proliferation. The proliferation was shown as Relative Luminescent Unit (RLU).

*Neutralization of human IL-34 bioactivity*

The optimal concentration of the antibody required to neutralize hIL-34 activity is dependent on the cytokine amount, cell type and the type of assay. To measure the ability of the antibody to neutralize the bioactivity of hIL-34 or mL-34 on PBMC, we used a cell proliferation assay by CellTiter-Glo. Based on cell response to serial dilutions of IL-34, 100 ng/ml of IL-34 amount was selected for determining the antibody neutralizing activity. The Half Maximal Inhibitory Concentration (IC<sub>50</sub>) is defined as the concentration of antibody required to yield half maximal inhibition of IL-34 activity on cells, when IL-34 is present at a concentration to elicit 70-80% proliferation response. 100 ng/ml hIL-34 or mL-34 was combined with serial dilutions of anti-IL-34 mAbs or anti-CSF1 antibodies before adding onto cells in a total

volume of 100 ul. The antibody inhibition activity was obtained by measuring RLU after incubating the plates at 37 °C for 72 hours. IC50 values were calculated with KaleidaGraph.

*Antibody generation by phage display*

*Library Sorting and Screening to Identify Anti-IL-34 Antibodies*

5 Murine IL-34 (PRO307278) was used as antigen for library sorting. Nunc 96 well Maxisorp immunoplates were coated overnight at 4°C with target antigen (10µg/ml) and were blocked for 1 hour at room temperature with phage blocking buffer PBST (phosphate-buffered saline (PBS) and 1% (w/v) bovine serum albumin (BSA) and 0.05% (v/v) tween-20). Human synthetic antibody phage libraries VH (see, e.g., Lee et al., *J. Immunol. Meth.* 284:119-132, 10 2004) and VH/VL (see Liang et al., *Journal of molecular biology* 366: 815-829 (2007)) with synthetic diversities in the selected CDRs were added to antigen plates separately and incubated overnight at room temperature. The following day antigen-coated plates were washed ten times with PBT (PBS with 0.05% Tween-20), and bound phage were eluted with 50mM HCl and 500mM NaCl for 30 minutes and neutralized with an equal volume of 1 M 15 Tris base (pH7.5). Recovered phages were amplified in *E. coli* XL-1 Blue cells. During the subsequent selection rounds, incubation of antibody phage with the antigen-coated plates was reduced to 2-3 hours, and the stringency of plate washing was gradually increased. After 4 rounds of panning, significant enrichment was observed. 96 clones were picked each from VH and VH/VL library sorting to determine whether they were human/murine IL-34 20 cross or murine IL-34 specific binders. The variable regions of these clones were PCR sequenced to identify unique sequence clones. These unique phage clones were subsequently reformatted into IgGs by cloning VL and VH regions of individual clones into the LPG3 and LPG4 vector respectively (Carter et al., *Proc. Natl. Acad. Sci. USA*, **89**: 4285-4289 (1992)), transiently expressing in mammalian CHO cells, and purifying with a protein A column.

25 *Construct Libraries for Affinity Improvement of YW404.33*

Phagemid pW0703 (derived from phagemid pV0350-2b (Lee, 2004) displaying monovalent Fab on the surface of M13 bacteriophage) served as the library template for grafting light (VL) and heavy (VH) chain variable domains of YW404.33 for affinity maturation. Stop codons were then incorporated in CDR-L3 of the library template. Soft randomization 30 strategy was used for affinity maturation, which introduced a mutation rate of approximately 50% at the selected positions by using a poisoned oligonucleotide strategy with 70-10-10-10 mixtures of bases favoring the wild type nucleotides (Gallop et al., *Journal of medicinal*



*chemistry* **37**: 1233-1251 (1994)). Specifically residues at positions 28-32 of CDR-L1, 50 and 53-55 of CDR-L2, 91-94 and 96 of CDR-L3, 28-35 of CDR-H1, 50-58 of CDR-H2, 95-100 of CDR-H3, were targeted. And three different libraries with combinations of soft-randomized CDR loops, L1/L2/L3, L3/H1/H2 and L3/H3, were constructed.

#### 5           *Phage Sorting Strategy to Generate Affinity Improvement*

For affinity improvement selection, phage libraries were subjected to plate sorting for the first round, followed by four rounds of solution sorting. At the first round of plate sorting, three libraries were sorted against murine IL-34 coated plate (NUNC Maxisorp plate) for 2 hours at room temperature. Next, four more rounds of solution sorting were carried out together with  
10 two methods of increasing selection stringency. The first of which is for on-rate selection by decreasing biotinylated target protein concentration from 50 nM to 0.5 nM, and the second of which is for off-rate selection by adding excess amounts of non-biotinylated target protein (100~500 fold more) to compete off weaker binders at room temperature. Each phage library was also incubated with non-biotinylated mIL-34 to serve as background phage binding for  
15 estimating the enrichment of each round of panning.

#### *High Throughput Affinity Screening ELISA (Single Spot Competition)*

After five rounds of panning, a high-throughput single-point competitive phage ELISA was used to rapidly screen for high-affinity clones as described (Sidu, 2004). Clones with low binding ratio to immobilized mIL-34 in the presence of 5nM mIL-34 verse in the absence of  
20 mIL-34 were chosen for further characterization.

#### *Affinity Measurement*

An Octet QK equipped with streptavidin (SA) biosensor tips (fortéBio, Menlo park, CA, USA) was used for affinity measurement. SA biosensor tips were equilibrated in assay buffer (1x Kinetics assay buffer, fortéBio) for 10min prior to analysis. Binding kinetics were  
25 measured at 300C and samples were agitated @ 1000 rpm. SA tips were saturated with 5ug/ml of biotinylated human IL-34 (R&D, Cat #5265-IL-010/CF) for 200s, which resulted in capture levels of  $0.22 \pm 0.02$  nm within a column of six tips. Three fold Serial dilutions of anti-IL-34 antibodies (100nM, 33.3nM, 11.1nM, 3.7nM, 1.2nM), as well as buffer blank were prepared. Both association and dissociation were monitored for 300s. All reactions and  
30 measurements were performed at room temperature. Data were analyzed using Octet data analysis software 6.4 (fortéBio).

## Results

Herein the first crystal structure of human IL-34 alone and in complex with CSF-1R is presented, and their binding properties in solution were characterized. It is shown that IL-34 indeed belongs to the short-chain helical cytokine family, with the smallest dimerization interface among the family members. The structure of the IL-34/CSF-1R complex showed a similar overall ligand/receptor assembly in terms of the domains involved, as was seen for the CSF-1 complex. However, the receptor domains involved undergo an unexpected rearrangement, resulting in different interface compositions. A careful comparison between these two cytokine signaling complexes provided an understanding of the molecular determinants that allow CSF-1R to promiscuously bind two structurally-related, yet evolutionarily-distant ligands IL-34 and CSF-1. Furthermore, co-crystal structures of IL-34 in complex with Fab fragments of two phage-derived anti-IL-34 monoclonal antibodies provided a structural rationale for their respective non-blocking and neutralizing activities, and offer novel insights into therapeutic development.

### Structure of the active core of human IL-34

The mature full-length human IL-34 is comprised of 222 amino acids, but its last ~50 residues are predicted to be largely disordered with little discernable secondary structure by Jpred 3(Cole et al., *Nucleic acids research* **36**: W197-201 (2008)) and PsiPRED (McGuffin et al., *Bioinformatics* **16**: 404-405 (2000)). Moreover, constructs of IL-34 containing the first 202 or 182 residues were indistinguishable from the full-length protein in activating the growth of TF-1-fms cells, while constructs encompassing only the first 162 residues showed significantly diminished activity (Chihara et al., *Cell death and differentiation* **17**: 1917-1927 (2010)). In addition, seven cysteine residues (35, 168, 177, 179, 180, 191, and 199) were found in human IL-34 and six of them (all but C199) are well-conserved across species (Figure 1A, Figure 7). Therefore, a truncated IL-34 construct comprised of residues N21–V193 of the mature polypeptide, herein referred to as hIL-34s, was chosen for the subsequent studies. hIL-34s was fused to a C-terminal flag-tag, expressed recombinantly in insect cells, purified as described in the methods, and used in all subsequent studies, unless otherwise noted. hIL-34s was as active as CSF-1 and slightly more active than recombinant full-length IL-34 in its ability to promote human monocyte viability (Figure 1B). The human IL-34 gene contains no recognizable membrane-associating regions, while the mouse ortholog exists

naturally as GPI-anchored and soluble isoforms as a result of alternative RNA splicing and proteolytic processing (Figure 1A).

The structure of the active core domain of human IL-34 was determined by molecular replacement from its complex with a non-blocking antibody YW405.3 Fab fragment (Table 6) at 2.6 Å resolution, and refined using a 1.85 Å resolution dataset collected from a single hIL-34s crystal (Table 2). Not surprisingly, structure analysis indicated that IL-34 has the distinctive antiparallel four-helix bundle cytokine fold consisting of  $\alpha$ A,  $\alpha$ B,  $\alpha$ C, and  $\alpha$ D (Sprang et al., *Curr Opin Struc Biol* **3**: 815-827 (1993)), but the structure contains a number of notable features outside this core portion (Figure 1C). The crossing  $\beta$ -strands 1 and 2 are much shortened and partially substituted by three short helices ( $\alpha$ 1,  $\alpha$ 2, and  $\alpha$ 3) compared to CSF-1 (Chen et al., *Proceedings of the National Academy of Sciences of the United States of America* **105**: 18267-18272 (2008); Pandit et al., *Science* **258**: 1358-1362 (1992)) (Figure 1D). Furthermore, two intramolecular disulfide pairs located at the pole of each protomer, and away from the dimer interface, share no structural similarities with disulfide bonds found in the structures of the related dimeric “short-chain” helical cytokines CSF-1, SCF, and Flt3L (Jiang et al., *The EMBO journal* **19**: 3192-3203 (2000); Savvides et al., *Nature structural biology* **7**: 486-491 (2000); Zhang et al., *Proceedings of the National Academy of Sciences of the United States of America* **97**: 7732-7737 (2000)). The first disulfide bond (between C35 and C180) connects helices  $\alpha$ A and  $\alpha$ D, while the other disulfide bond (between C177 and C191) connects  $\alpha$ D to the C-terminal helix  $\alpha$ 4. Consequently, the C-terminal tail including  $\alpha$ 4 is inverted, compared with that of CSF-1, and packs onto the surface of  $\alpha$ A and  $\alpha$ D. The other two cysteines C168 and C179 remain unpaired and consequently are not essential for the proper folding of IL-34. Overall, as judged by PDBeFOLD structural superposition (Krissinel et al., *Acta crystallographica Section D, Biological crystallography* **60**: 2256-2268 (2004)), IL-34 was shown to be structurally most similar to SCF (root-mean-square deviation (rmsd): 2.6 Å), yet more divergent from the functionally related CSF-1 (rmsd: 3.2 Å).

The results of this structure analysis also showed that the two protomers of IL-34 in the asymmetric unit further assemble into a non-covalent dimer in a manner similar to that seen for CSF-1, SCF, and Flt3L. In forming the IL-34 dimer, each subunit buries 656 Å<sup>2</sup>, with the  $\alpha$ A- $\beta$ 1 loop and  $\alpha$ B- $\alpha$ C loop from one monomer interlocking with the reciprocal segments of the other monomer. A compact and relatively flat hydrophobic patch centered on P58/P58' is formed by packing the side chains of H56, Y57, F58, P59, and Y62 of one protomer against residues P114', H113', L110', L109', V108', Y62', P59', and F58' from the neighboring

monomer. These interactions may confer obligate IL-34 dimer formation despite the smaller buried surface area (1310 Å<sup>2</sup>) compared with the related non-covalent SCF and Flt3L dimers, comprised of 1690 Å<sup>2</sup> and 1640 Å<sup>2</sup>, respectively. The residues at the IL-34 dimer interface are highly conserved among orthologs from other species (Figure 7). The dimeric

5 organization of IL-34 observed in hIL-34s crystals is likely to be representative of the organization of the protein in solution as the same “head-to-head” dimeric arrangement was also present in the three IL-34 complex structures discussed herein. One predicted N-linked glycosylation site in mature IL-34 located at N76 was included in the construct that was crystallized, and electron density for (GlcNAc)<sub>2</sub>Man was observed attached to the side chain.

10 The results indicated that orientation of the carbohydrate is fixed by stacking interactions between the aromatic ring of Y68 and the hydrophobic face of the second GlcNAc residue, together with polar interactions mediated by three conserved residues in IL-34, E69, R79, and K157, suggesting that this sugar moiety is an integral part of the IL-34 structure. The analogous glycosylation site is conserved in rodent IL-34, which also contains an additional  
15 predicted N-linked glycosylation site at a position equivalent to human S100, which is solvent accessible in the hIL-34s structure.

#### *Biophysical characterization of the IL-34/CSF-1R complex*

To probe the oligomeric state of hIL-34s in solution, analytical size exclusion chromatography was used (Figure 2A). The theoretical molecular mass for the hIL-34s  
20 monomer is 22 kDa, yet the protein eluted earlier than the 44kDa reference, indicating hIL-34s forms dimers in solution, in agreement with the two-fold non-crystallographic symmetric dimer structure observed in the hIL-34s crystal. In order to assess the molecular size of IL-34 ligand/receptor complexes, a construct containing nearly the entire extracellular domain (ECD) including immunoglobulin domains D1 through D5 of the receptor (hCSF-1R D1-D5)  
25 was mixed with IL-34 at 1:1 protomer molar ratios and analyzed by the same technique. The apparent size of this complex was consistent with the size calculated for a 2:2 complex (Figure 2A). Similarly, in order to narrow down the minimal receptor construct necessary for complex formation, a mixture of hIL-34s with a receptor construct containing only the first three immunoglobulin domains (hCSF-1R D1-D3) was subject to size exclusion analysis,  
30 revealing an apparent size which is larger than 158 kDa, in agreement with a 2:2 complex (Figure 2A).

To accurately determine the stoichiometry and energetics of IL-34 binding to CSF-1R and compare them with CSF-1 binding, the thermodynamic parameters for binding were assessed by isothermal titration calorimetry (ITC) (Figure 2B, D). Titrating CSF-1R receptor into the IL-34 cytokine confirmed that both the full-length and truncated forms of the receptor, CSF-1R D1-D5 and D1-D3, were saturated by equal molar amounts of IL-34 monomer. This, in conjunction with the apparent sizes determined by analytical size exclusion chromatography, strongly supported a 2:2 cytokine/receptor stoichiometry in solution for the complex (Figure 2D). Since the high affinity of the IL-34/CSF-1R D1-D5 interaction yielded a steep titration transition that precluded determining the dissociation constant with high accuracy (Figure 2B, left panel), displacement ITC was carried out by titrating CSF-1R D1-D5 into a mixture of IL-34 with CSF-1R D1-D3 (Figure 2B, right panel). The binding isotherms determined herein indicated IL-34 binding to its receptor is driven mainly by enthalpic terms. The exothermic nature of IL-34 binding to both CSF-1R D1-D3 and D1-D5 indicated that IL-34/CSF-1R complex formation likely involves key polar interactions. Inclusion of the membrane-proximal domains CSF-1R D4-D5 lead to a large increase in affinity (1.6 and 94 nM  $K_d$  for D1-D5 and D1-D3, respectively), which is significantly more favorable enthalpically, although slightly disfavored entropically. These results suggested that although hCSF-1R D1-D3 is sufficient to confer high-affinity ligand-binding, CSF-1R D4-D5 likely contains additional homotypic receptor interaction sites upon formation of the entire cytokine/receptor signaling complex, as seen in the complex structure of SCF bound to the full ectodomains of a KIT receptor dimer (Yuzawa et al., *Cell* **130**: 323-334 (2007)). Surprisingly, the better characterized ligand CSF-1 exhibited 9.5 and 13-fold lower affinity compared to IL-34 for binding to hCSF-1R D1-D3 and D1-D5, respectively (Figure 2C, D). Compared to IL-34, the results indicated that CSF-1 complexes are driven by both favorable enthalpy and entropy. Similar to IL-34, the human CSF-1 titrations also suggested 2:2 ligand/receptor stoichiometries for both forms of the receptors. Although consistent with an earlier report (Guilbert et al., *The Journal of biological chemistry* **261**: 4024-4032 (1986)), this represents a key difference between human and murine CSF-1. In the absence of D4 and D5, murine CSF-1 was reported to form a 2:1 partial complex with murine CSF-1R leaving half of the receptor binding surface on murine CSF-1 unoccupied in solution (Chen et al., *Proceedings of the National Academy of Sciences of the United States of America* **105**: 18267-18272 (2008)). To further understand the kinetics of cytokine/receptor binding in this system, biotinylated IL-34 or CSF-1 were immobilized on streptavidin-coated biosensor tips in order to measure rates

of association and dissociation with serial receptor dilutions using the Bio-Layer Interferometry (BLI) technique. The results from this orthogonal method also confirm that IL-34 ( $k_{\text{on}} = 2.9 \times 10^5 \text{ s}^{-1}\text{M}^{-1}$ ,  $k_{\text{off}} = 3.5 \times 10^{-2} \text{ s}^{-1}$ ,  $K_d = 120 \text{ nM}$ ) binds CSF-1R more tightly than CSF-1 ( $k_{\text{on}} = 7.7 \times 10^4 \text{ s}^{-1}\text{M}^{-1}$ ,  $k_{\text{off}} = 5.4 \times 10^{-2} \text{ s}^{-1}$ ,  $K_d = 720 \text{ nM}$ ) primarily due to a 3.8-fold faster  $k_{\text{on}}$  coupled to a slightly slower  $k_{\text{off}}$ .

#### *Structure of the IL-34/CSF-1R D1-D3 complex*

The 3.0 Å resolution structure of hIL-34s bound to hCSF-1R D1-D3 was solved by molecular replacement using structures of hIL-34s and murine CSF-1R (Chen et al., *Proceedings of the National Academy of Sciences of the United States of America* **105**: 18267-18272 (2008)) as search probes (Table 2). Although all biophysical methods tested in this study indicated that hCSF-1R D1-D3 forms a 2:2 complex with hIL-34s in solution, the crystal structure unambiguously revealed one CSF-1R D1-D3 bound to one hIL-34s homodimer in the asymmetric unit. Structure analysis suggested that the analogous receptor binding site on the adjacent protomer is involved in crystal packing, prohibiting formation of the expected 2:2 complex in this crystal structure form. However, this structure nicely revealed the IL-34/receptor interface. It showed that IL-34 is bound to a concave surface formed by D2 and D3 of CSF-1R in a configuration resembling that employed by CSF-1 when bound to the CSF-1R receptor. A similar two-site binding mode seen in the CSF-1 cytokine/receptor complex is also preserved in the IL-34/CSF-1R interface. These results indicated that Site 1 involved interactions between IL-34 and Ig domain D2 of the receptor, and Site 2 between IL-34 and receptor domain D3. Each site provides 1280 Å<sup>2</sup> and 1160 Å<sup>2</sup> total buried surface area, respectively. The linker between D2 and D3, although fully ordered in the structure, is spared from the interaction with IL-34, which effectively parses the IL-34/CSF-1R interface into two spatially separated sites.

#### *IL-34 Ligand/receptor Binding Site 1*

Structure analysis revealed that Site 1 is formed mainly by receptor D2 domain residues comprising the CD and EF loops (residues 142-150 and 169-173, respectively) which dock onto the rugged surface provided by IL-34 helices αB (residues 100-108), αC (116-134), the intervening loop (residue 109), and α3 (residue 150) (Figure 3A). Complimentary electrostatic interactions between the negatively-charged surface of IL-34 and the positively-charged surface on CSF-1R in this region appeared important in mediating IL-34 binding at this site. In particular, centrally located salt bridges are formed between the basic amino acids

R142, R146 of CSF-1R with the acidic E103 on IL-34. R144 and R150 of CSF-1R at the periphery of the CD loop engage in hydrogen-bonding interactions with the side chain oxygen of N150 and the backbone carbonyl oxygen of Q106 and L109, respectively. A small hydrophobic patch formed by the aliphatic side chain of L127 in IL-34 fits snugly between F169 and I170 of CSF-1R. While this site appeared dominated by polar interactions, a number of intermolecular van der Waals contacts also line the interface as detailed in Table 3.

#### *IL-34 Ligand/receptor Binding Site 2*

The results further indicated that the slightly smaller Site 2 is formed mainly by receptor D3 residues from the BC and DE loops (residues 231 and 254, respectively) and strand D (residues 248-252), packed against the surface generated by portions of IL-34 helices  $\alpha$ A,  $\alpha$ C, and  $\alpha$ 4 (residues 36-44, 121-128, 184-187, respectively) (Figure 3C). This interface can be further divided into two polar interaction regions, separated by a hydrophobic area formed by IL-34 residues F40 and L125, and CSF-1R residues V231, Y257, and F252. The first region is formed by a combination of backbone and side chain hydrogen-bonding interactions between the beginning of the CSF-1R D3 D-strand and IL-34  $\alpha$ 4. The side chain amide hydrogen and oxygen of N187 of IL-34 participates in up to three hydrogen bonds with the receptor involving the side chain amide of Q249, and the backbone nitrogen and carbonyl oxygen of S248 of CSF-1R. Additionally, two backbone-backbone hydrogen bonds are formed between CSF-1R Q248 and IL-34 S184 and L186. Deletion of residues comprising the  $\alpha$ 4 region in IL-34 results in drastic reduction in protein expression levels, and significantly weaker activity in terms of the ability to stimulate the growth of TF-1-fms cells (Chihara et al., *Cell death and differentiation* **17**: 1917-1927 (2010)). The results reported here confirmed that residues encompassing  $\alpha$ 4 are an integral part of the active core domain of IL-34, and are therefore important for its activity. The second polar region in Site 2 encompasses three side-chain-mediated hydrogen bonds between the terminal side chain atoms of IL-34 N128, K44 and E121, and the hydroxyl group of Y257, the carbonyl oxygen of F252 and the backbone amide of N254 of CSF-1R. Additional interactions mediated by van der Waals contacts are listed in Table 3.

Table 3. IL-34/CSF-1R and CSF-1/CSF-1R interactions.

CSF-1R D2		IL-34		Distance (Å)	CSF-1		Distance (Å)
Hydrogen bonds and salt bridges (Shared)							
Arg142	Nn1				Gln58	Oε1	3.4

	Nη2	Glu103	OE1	3.1			
	Nη2	Glu103	OE2	3.2			
Arg146	Nη1				Gln58	Oε1	2.4
	Nη2	Glu103	OE1	2.6	Asp59	Oδ1	3.4
Arg150	Nη1	Leu109	O	3.2			
	Nη2	Gln106	O	3.5	Asp62	O	3.0
	N				Asp62	Oδ1	3.4
	N				Asp62	Oδ2	3.2
Hydrogen bonds and salt bridges (Unique)							
Arg144	Nη1	Asn150	Oδ1	2.5			
Lys151	Nζ				Glu78	Oε1	3.9
Lys168	Nζ				Glu78	Oε1	3.6
Lys168	Nζ				Glu78	Oε2	3.7
Leu170	O				Asn85	Nδ2	3.1
van der Waals contacts (Shared)							
Arg146		Ser100, Asn150			Phe55		
Met149 (Leu149)		Glu123			Asp62, Gln81		
Arg150		Trp116			Arg66		
Phe169 (Val169)		Thr124, Leu127, Asn128, Gln131			Glu82		
Ile170 (Leu170)		Glu123, Thr124, Leu127			Glu78		
Gln173 (Asn173)		Leu127			Glu78		
van der Waals contacts (Unique)							
Lys151					Asp62		
Ser172		Leu127					
Arg192		Thr134					
CSF-1R D3		IL-34		Distance (Å)	CSF-1		Distance (Å)
Hydrogen bonds and salt bridges (Shared)							
Tyr257	OH	Asn128	Nδ2	2.7	Met10	O	2.6
Hydrogen bonds and salt bridges (Unique)							
Gln248	N	Ser184	O	2.7			
	O	Leu186	N	2.8			
Gln249	Nε2	Asn187	Oδ1	3.1			
Ser250	N	Asn187	Oδ1	3.0			
	O	Asn187	Nδ2	3.5			
Asp251	Oδ1				Asn13	N	3.0
	Oδ2				Gly14	N	2.9
Phe252	O	Lys44	Nζ	2.6			
Asn254	N	Glu121	Oε2	3.8			
van der Waals contacts (Shared)							
Val231		Phe40			His6, Met10		
Ser250		Phe40, Asp43			His9		
Asp251		Phe40, Asp43			Gly12		
Phe252		Leu125			Gly14, His15, Arg79		
Tyr257		Phe40, Leu125			His9, His15		
van der Waals contacts (Unique)							



Gly232		His6
Phe233		His6
Pro247	Ser184, Gln189	
Gln248	Thr36, Val185, Asn187	
Gln249	Leu186	
Asn255		Arg79
Gln258	Phe40	
Lys259	Thr36	

#### *Structural basis for IL-34 antagonism by mAb YW404.33.56*

To dissect the discrete functions of IL-34 and CSF-1 and explore the therapeutic benefit from blocking IL-34 signaling, phage display technology was used to generate antibodies that specifically antagonize both human and murine IL-34, enabling the interrogation of IL-34 function in both human patients and rodent models. YW404.33 was identified by screening of  $V_H$  and  $V_H/V_L$  phage display libraries and selected out of a panel of 96 clones based on its high binding affinity ( $K_d = 17$  nM, Table 4) and its blocking activity for human IL-34. YW404.33 was subsequently affinity-matured using a soft randomization strategy as described in the Methods. YW404.33.56 shows a 140-fold improvement in binding affinity ( $K_d = 120$  pM) as measured by BLI experiments when compared with the parental clone. In human monocyte viability (Figure 4A) and proliferation (Figure 8) assays, the biological activity of IL-34 was completely blocked by this antibody, similar to the receptor Fc fusion, but not by an anti-CSF-1 antibody.

Table 4. Octet measurement of binding kinetics for the non-neutralizing antibody YW405.3/hIL-34, and neutralizing antibodies YW404.33/hIL-34, YW404.33.56/hIL-34.

Anti-IL-34 Mabs	$k_{on}(s^{-1}M^{-1})$	$k_{off}(s^{-1})$	$K_d(M)$
YW405.3	1.45E+06	4.52E-03	3.12E-09
YW404.33	3.58E+05	6.11E-03	1.71E-08
YW404.33.56	3.38E+06	4.19E-04	1.24E-10

To better understand the mechanism whereby YW404.33.56 mAb blocks IL-34 biological activity, the structure of hIL-34s in complex with the Fab fragment of YW404.33.56 was solved at 3.0 Å resolution (Table 2). Crystals contained one hIL-34s dimer bound to two YW404.33.56 Fabs in the asymmetric unit. Interestingly, structure analysis showed that each YW404.33.56 Fab recognizes a largely continuous area at the junction of two IL-34 protomers. On the IL-34 dimer side, the majority of the interface (786 Å<sup>2</sup> or 79%) is contributed by helices  $\alpha B$ ,  $\alpha C$ , and their intervening loop, from one protomer, whereas a

smaller fraction (215 Å<sup>2</sup> or 21%) is contributed by the αB-β1 loop of the other IL-34 protomer (Figure 4B). On the YW404.33.56 Fab side, the bulk of the interactions are mediated by the heavy chain CDR-H1 (55 Å<sup>2</sup>), CDR-H2 (267 Å<sup>2</sup>), and CDR-H3 (316 Å<sup>2</sup>) with smaller contributions from the light-chain CDRs (303 Å<sup>2</sup>). Four salt bridges (R50<sup>YW404.33.56</sup>-E111<sup>IL-34</sup>, R100b<sup>YW404.33.56</sup>-E111<sup>IL-34</sup>, R100b<sup>YW404.33.56</sup>-D107<sup>IL-34</sup>, and K100a<sup>YW404.33.56</sup>-E103<sup>IL-34</sup>) form an “electrostatic zipper” along the groove between αB and αC of IL-34. This strong electrostatic complementarity between IL-34 and YW404.33.56 Fab was reminiscent of the Site 1 charge-charge interactions in the IL-34/CSF-1R complex described above. Additionally, W33<sup>YW404.33.56</sup>, Y54<sup>YW404.33.56</sup>, K98<sup>YW404.33.56</sup>, and S100<sup>YW404.33.56</sup> form side chain specific hydrogen bonds with D107<sup>IL-34</sup>, D107<sup>IL-34</sup>, S104<sup>IL-34</sup>, and Q120<sup>IL-34</sup>, respectively. In addition, other YW404.33.56 backbone carbonyl groups mediate hydrogen bonds, and several other van der Waals interactions as detailed in Table 5. The carbohydrate chain of the glycosylated N76 of IL-34 extends away from the interaction interface, and has no direct contact with the YW404.33.56 Fab. Unexpectedly, hydrophobic packing across the interface, which often mediates high-affinity protein-protein interactions, is absent in this antibody/antigen system. The shape complementarity between the Fab paratope and IL-34 epitope was 0.61, which is slightly smaller than the average value (0.64–0.68) for other antibody/antigen complexes (Lawrence et al., *Journal of molecular biology* **234**: 946-950 (1993)), suggesting the paratope of YW404.33.56 mAb could in theory be further optimized to achieve higher shape and chemical complementarities for tighter binding to IL-34.

Superposition of the IL-34 molecules in the IL-34/YW404.33.56 and IL-34/CSF-1R complexes showed that both the heavy and light chains of the Fab clash with CSF-1R. These results indicated that YW404.33.56 binds to an epitope that overlaps largely with binding epitopes responsible for Site 1, CSF-1R D2-binding, and includes residues from helices αB and αC from one IL-34 protomer. 6 out of 11 residues or 220 Å<sup>2</sup> out of 600 Å<sup>2</sup> of the buried solvent-accessible surface areas from IL-34 at the IL-34/CSF-1R binding Site 1 are occupied by interactions with the YW404.33.56 in the IL-34/Fab complex. Therefore, the binding of YW404.33.56 to IL-34 would compete directly for association with the receptor CSF-1R, which explains the molecular mechanism of the inhibition of IL-34 signaling by YW404.33.56.

Table 5. IL-34/YW404.33.56 Fab interactions.

YW404.33.56 H chain		IL-34 mol A		Distance (Å)
Hydrogen bonds and salt bridges				
Trp33	Nε1	Asp107	Oδ1	3.5
	Nε1	Asp107	Oδ2	3.4
Arg50	Nη1	Glu111	Oε2	3.0
Arg50	Nη2	Glu111	Oε1	3.0
Tyr54	OH	Asp107	Oδ2	3.0
Lys98	Nζ	Ser104	Oγ	3.5
Gly99	N	Asp107	Oδ1	2.4
Ser100	Oγ	Gln120	Oε1	3.0
Lys100A	Nζ	Glu103	Oε1	2.8
Arg100B	Nε	Glu111	Oε2	3.7
Arg100B	Nη1	Asp107	Oδ1	3.4
Arg100B	Nη1	Asp107	O	2.9
Arg100B	Nη2	Leu109	O	2.8
Arg100B	Nη2	Glu111	Oε1	3.9
Arg100B	Nη2	Glu111	Oε2	3.2
van der Waals contacts				
Thr28		Pro152		
Tyr54		Val108		
Tyr56		Leu110		
Lys98		Glu103, Asp107		
Gly99		Gln106, Trp116		
Ser100		Gln106, Trp116		
Lys100A		Glu123		
Arg100B		Leu109, Leu110, Trp116		
YW404.33.56 L chain		IL-34 mol A		Distance (Å)
Hydrogen bonds and salt bridges				
Phe92	O	Trp116	Nε1	3.3
van der Waals contacts				
Phe53		Leu127		
Ser91		Trp116		
Phe92		Glu111, Lys117, Gln120		
Phe94		Glu111		
YW404.33.56 H chain		IL-34 mol B		Distance (Å)
Hydrogen bonds and salt bridges				
Tyr54	O	Asn61	Nδ2	2.7
Tyr55	O	Asn61	Nδ2	3.4
van der Waals contacts				
Trp33		Ile60		
Tyr54		Ile60		
Try56		Lys55, Ile60		

Table 6. IL-34/YW405.3 interactions

YW405.3 H chain		IL-34 mol A		Distance (Å)
Hydrogen bonds and salt bridges				
Trp52A	O	Arg170	Nη2	3.2
Tyr53	O	Arg170	Nε	2.7
	OH	Asn167	Oδ1	2.3
Gly54	O	Lys162	Nζ	2.8
Gly55	O	Tyr47	OH	2.7
Asp56	Oδ1	Lys162	Nζ	3.8
	Oδ1	Tyr47	OH	3.7
	Oδ2	Lys55	Nζ	3.6
Asp98	Oδ1	Lys63	Nζ	2.7
	Oδ1	Arg160	Nη1	2.6
	Oδ2	Lys63	Nζ	3.7
	Oδ2	Arg160	Nη1	3.2
van der Waals contacts				
Tyr53		Arg73, Ile74		
Gly54		Tyr47, Asp166		
Tyr99		Arg73		
YW405.3 L chain		IL-34 mol A		Distance (Å)
Hydrogen bonds and salt bridges				
Tyr32	OH	Leu158	O	3.5
Trp92	O	Arg160	Nη1	3.8
Glu94	N	Asn61	Oδ1	3.5
van der Waals contacts				
Tyr32		Lys63		
Trp92		Asn61, Lys63, Leu158		
Ser93		Asn61		
YW405.3 L chain		IL-34 mol B		Distance (Å)
Hydrogen bonds and salt bridges				
Ser26	O	Asn155	N	2.9
van der Waals contacts				
Ala25		Pro154		
Ser26		Pro154		

## 5 Discussion

### *Conformational change in IL-34 upon binding to the receptor*

Herein the first three-dimensional structures of IL-34, solved both on its own and in complex with its receptor CSF-1R are reported. Comparison of the IL-34 protomer structure in its

unbound and CSF-1R-bound forms revealed that the structure of the cytokine is nearly unchanged upon binding to its receptor. Even the side chain rotamer conformations adopted by the receptor interfacial residues showed surprisingly little difference between free and bound states, indicating the unbound IL-34 protomer is highly compatible with, and primed for receptor-binding. The apparent rigidity of the IL-34 protomer structure based on comparisons of the four structures presented herein was consistent with the recently determined Flt3L structure (Verstraete et al., *Blood* 118: 60-68 (2011)), but quite different from the more plastic CSF-1 (Chen et al., *Proceedings of the National Academy of Sciences of the United States of America* **105**: 18267-18272 (2008)) or SCF (Liu et al., *The EMBO journal* **26**: 891-901 (2007); Yuzawa et al., *Cell* **130**: 323-334 (2007)) structures, which must undergo significant local structural changes to accommodate receptor-binding. Nevertheless, significant changes in the orientation of one IL-34 protomer relative to the other protomer about the dimer interface upon complex formation were observed. When described by the tilt angle of  $\alpha C$ , receptor-binding or YW404.33.56-binding induced a 6.6° or 4.6° increase in the angles between IL-34 protomers, respectively. Similar hinge-like rigid-body movements were reported previously in the CSF-1/CSF-1R, SCF/Kit and Flt3L/Flt3 systems. Unexpectedly, another antibody YW405.3 triggered a similar rotation, but along the reverse direction, resulting in 6.4° decrease in the tilt angle. Such high degree of plasticity is unprecedented in other dimeric four helical bundle cytokines, and can likely be attributed to the smaller, and very hydrophobic IL-34 dimerization interface.

#### *The dual recognition of IL-34 and CSF-1 by CSF-1R*

While IL-34 and CSF-1 are sparsely similar at the primary sequence level, it was found that they indeed adopt a similar four helical bundle core fold and related dimerization and receptor binding interfaces, with differences apportioning to extra-core loops and structural embellishments - a recurrent finding in the comparison of helical cytokine structures (Bazan, *Neuron* **7**: 197-208 (1991b); Hill et al., *Journal of molecular biology* **322**: 205-233 (2002); Rozwarski et al., *Structure* **2**: 159-173 (1994)). In fact, a telling remnant of helical cytokine ancestry (aside from the three-dimensional fold relationship) is the similarity in exon/intron structures of their respective genes (Bazan, *Cell* **65**: 9-10 (1991a); Betts et al., *The EMBO journal* **20**: 5354-5360 (2001)); in this respect, the structure of the IL-34 gene is homologous to the CSF-1, SCF and Flt3L genes.

Both IL-34 and CSF-1 bound the CSF-1R receptor with high-affinity and induced similar, if not identical, biological activity (Chihara et al., *Cell death and differentiation* **17**: 1917-1927 (2010); Lin et al., *Science* **320**: 807-811 (2008)). This striking phenomenon lead to the direct comparison of the structure of the human IL-34/CSF-1R complex to that of the murine CSF-1/CSF-1R complex to determine the molecular mechanisms governing receptor sharing by these structurally similar but evolutionally divergent ligands. This analysis indicated that, CSF-1R utilizes a common “dual interface mode” for its interactions with both cytokines. The total solvent-accessible surface area buried at the interface between IL-34 and CSF-1R is approximately 2400 Å<sup>2</sup>, significantly larger than the 1700 Å<sup>2</sup> buried in the CSF-1/CSF-1R interface. Notably, the regions of CSF-1R that interact with IL-34 and CSF-1 largely overlap, yet are not identical. Consistent with this result, the anti-CSF-1R mAb clone 12-2D6 that blocked signaling by both cytokines (Chihara et al., *Cell death and differentiation* **17**: 1917-1927 (2010)), recognizes an epitope between residues 1-308 within the first three domains of the CSF-1R receptor. 12-2D6 most likely functions by binding to a site on the receptor overlapping the IL-34/CSF-1 binding sites, and therefore abrogates receptor signaling irrespective of the ligands. In contrast, mAb clone 2-4A5 blocked CSF-1, but not IL-34 binding to TF-1-fms cells by recognizing an epitope residing between residues 349-512 (Chihara et al., *Cell death and differentiation* **17**: 1917-1927 (2010)). Considering this epitope is remote from the ligand-binding sites of both cytokines, the ability of 2-4A5 to distinguish CSF-1 from IL-34 remains an enigma. Nevertheless, steric hindrance could be created by the specific geometry of this antibody/CSF-1R complex, which affects only CSF-1 binding.

Further analysis revealed substantial differences in both the numbers and types of interactions between the two CSF-1R signaling complexes. The sequences of human and mouse CSF-1R are more than 70% identical with no gaps within D1-D3; therefore allowing direct structural comparison of CSF-1R in the human IL-34 complex to that of the murine CSF-1 complex (Chen et al., *Proceedings of the National Academy of Sciences of the United States of America* **105**: 18267-18272 (2008)). The Site 1 interfaces share a number of common features. Most of the polar interactions between the CSF-1R CD loop and helix αB of both cytokines are mediated by the same group of basic residues on the receptor (R142, R146, and R150), which dictate the shared charge complementarity in this region (Figure 3A, B). Interestingly, these three residues on CSF-1R remain invariant while other shared interfacial contacts are more divergent among CSF-1R orthologs in other eukaryotic species. A centrally

located acidic residue (E103 in IL-34 and D59 in CSF-1) at the second turn of helix  $\alpha$ B participates in salt bridges in both structures. The hydrogen-bonding interactions between the EF loop of CSF-1R and N85 in CSF-1 are substituted by a nearby hydrophobic patch in the IL-34/CSF-1R complex. The most prominent differences are located at the edges of the CD and EF loops of CSF-1R. The salt bridges located at the lower edge of the two connecting loops between K151<sup>CSF-1R</sup>, K168<sup>CSF-1R</sup> and E78<sup>CSF-1</sup> are absent in IL-34/CSF-1R. Instead a unique hydrogen bond bridging N150<sup>IL-34</sup> and R144<sup>CSF-1R</sup> bring IL-34 into close contact with the upper edge of the CD loop. The differences between the Site 2 interfaces are even more striking. Wherein an extensive hydrogen-bonding network between the CD loop, D strand and DE loops of CSF-1R and helices  $\alpha$ A,  $\alpha$ C, and  $\alpha$ 4 in IL-34 forms the core of the interface at Site 2. Yet, such interactions are completely absent in the CSF-1/CSF-1R complex, resulting in a much smaller Site 2 interface. However, hydrophobic interactions involving V231 on the receptor were observed in both complexes.

*Large rearrangement in the CSF-1R receptor D2-D3 domain orientation in the IL-34 versus CSF-1 cytokine/receptor complexes*

The orientation of receptor domains D1 and D2 are conserved in both cytokine/receptor complexes; however the orientation of receptor domain D3 relative to D1-D2 showed a significant 27° change when comparing the two complexes. Engagement by IL-34 triggered a rotation between D2 and D3 of CSF-1R, producing an elongated, nearly linear pose that is significantly different from the kinked configuration of the CSF-1 bound form. This overall reorientation of CSF-1R could be attributed, at least in part, to the distinct IL-34 molecular surface, which would sterically clash with CSF-1R without inducing the new orientation observed in the structure reported herein. The receptor-contacting residues, when mapped onto the secondary structure of both cytokines clearly showed that both interaction sites are more spread out on IL-34 than CSF-1 (Figure 5A, B). This is mainly due to the distinct structural features outside the four-helix bundle core, namely,  $\alpha$ 3 and  $\alpha$ 4. Hence, a flatter interface is created on IL-34, requiring a more extended conformation of the receptor, while CSF-1 protrudes more into a cleft between CSF-1R D2 and D3 created from their more “bent” orientation. The interface between neighboring domains D2 and D3 is minimal in the CSF-1/receptor complex, yet the only salt bridge between D3 E230 and D2 K194 in that complex is broken in the IL-34/receptor complex, allowing D2 and D3 to adopt a more extended, almost linear arrangement upon IL-34 binding. The receptor D2-D3 hinge sequence

<sup>196</sup>NKVIPGP<sup>202</sup> completely reconfigures itself, using K197 and G201 as the two pivot points, while leaving D1-D2 ending with N196 and D3 starting with P202 with minimal structural perturbation. Thus, the substantial elbow flexibility between D2 and D3 domains allows the CSF-1R molecule to adapt to the distinct binding surfaces provided by IL-34 and CSF-1

(Figure 5A, B). In contrast, the receptor D1-D2-D3 region of KIT appeared to behave as a rigid body upon SCF binding, where D4 and D5 realign and mediate receptor-receptor interactions between the two SCF-bound KIT molecules (Yuzawa et al., *Cell* **130**: 323-334 (2007)). In Kit, there is a significant hydrophobic interface between domains D2-D3, which is absent in CSF-1R. Inter-domain flexibility has been used as an adaptive mechanism by which multiple viral proteins utilize the same host cell receptor for entry. Two structurally unrelated proteins, the measles virus hemagglutinin and adenovirus type 11/21 knobs share the first two SCR domains of CD46, the linker between which is quite structurally plastic (Cupelli et al., *Journal of virology* **84**: 3189-3200 (2010); Persson et al., *Nature structural & molecular biology* **14**: 164-166 (2007); Santiago et al., *Nature structural & molecular biology* **17**: 124-129 (2010)). Moreover, perturbation of receptor domain orientations can lead to pronounced functional consequences, as demonstrated by a 14° rotation in erythropoietin receptor (EPOR) in complex with synthetic agonist and antagonist peptide (Livnah et al., *Nature structural biology* **5**: 993-1004 (1998)). IL-34 was reported to induce a stronger but more transient activation of CSF-1R, and more rapidly downregulate CSF-1R levels compared to CSF-1 (Chihara et al., *Cell death and differentiation* **17**: 1917-1927 (2010)). Possibly this reorientation of CSF-1R receptor domains could modulate its signaling potency in combination with the different affinity to these two cytokines.

*Equally spaced CSF-1R D3-D4 junctions in the cytokine/CSF-1R signaling complexes prime D4, D5 for degenerative signaling*

Receptor-mediated homotypic interactions have been proposed to play an indispensable role in the activation of type III RTKs. Such interactions have been either captured structurally in the case of KIT D4 (Yuzawa et al., *Cell* **130**: 323-334 (2007)) and VEGFR2 D7 (Yang et al., *Proceedings of the National Academy of Sciences of the United States of America* **107**: 1906-1911 (2010)), or characterized biochemically in the case of PDGFRβ (Shim et al., *Proceedings of the National Academy of Sciences of the United States of America* **107**: 11307-11312 (2010)). The nature of these interactions is a pair of reciprocal salt bridges in the EF loop between the two receptor KIT D4 domains (D7 in VEGFR or D4 in PDGFR).



Due to the relatively high sequence identity between CSF-1R and KIT, punctuated by this conserved ionic pair in CSF-1R D4, similar receptor homotypic interactions probably also drive CSF-1R dimerization in relaying the ligand-binding signal across the membrane. Given the biophysical analyses described herein indicated a 2:2 stoichiometry of IL-34/CSF-1R complexes in solution, the 2:1 IL-34/CSF-1R complex observed in the crystal structure was most likely influenced by crystal packing forces. Thus, the expected full 2:2 ligand/receptor signaling complex was modeled by applying the 2-fold symmetry between two IL-34 protomers to CSF-1R. When the absent copy of CSF-1R was added to the unoccupied site on the IL-34 dimer, the distance (60 Å) between the two C termini of CSF-1R D3 in this 2:2 IL-34/CSF-1R model is very similar to that in the 2:2 CSF-1/CSF-1R model (62 Å), and to that in the 2:2 SCF/Kit structure (64 Å) (Figure 6). Taken together, although the hinge between CSF-1R D2-D3 can adopt very different conformations to adapt to distinct surface topographies of the two cytokines, the reorientation of D2-D3 nonetheless results in the D3-D4 junction being spaced equivalently in the two cytokine/receptor signaling complexes, presenting CSF-1 D4 as a convergent point for homotypic interactions to elicit degenerative responses downstream.

*Ligand-binding promiscuity in CSF-1R utilizes distinct mechanisms from shared hematopoietic cytokine receptors*

The molecular mechanism of cytokine-binding promiscuity have been deciphered for a number of shared cytokine receptors such as the common gamma chain ( $\gamma$ c), gp130 and interferon  $\alpha$  receptors (IFNAR1/2), since they all have been captured structurally in at least two different ligand-bound states (Thomas et al., *Cell* **146**: 621-632 (2011); Wang et al., *Annual review of immunology* **27**: 29-60 (2009)). The ectodomains of both  $\gamma$ c and gp130 contain one cytokine binding homology region (CHR) consisting of two Ig-like fibronectin type-III (FNIII) domains (Wang et al., *Annual review of immunology* **27**: 29-60 (2009)). Although the cytokine-binding site is housed at the interdomain junction in both shared class I cytokine receptors, no significant elbow movements have been observed by comparing the IL-2/ $\gamma$ c quaternary complex with IL-4/ $\gamma$ c ternary complex (LaPorte et al., *Cell* **132**: 259-272 (2008); Wang et al., *Science* **310**: 1159-1163 (2005)), or the unliganded gp130 with three gp130 family cytokine complexes (Boulanger et al., *Molecular cell* **12**: 577-589 (2003a); Boulanger et al., *Science* **300**: 2101-2104 (2003b); Bravo et al., *The EMBO journal* **17**: 1665-1674 (1998); Chow et al., *Science* **291**: 2150-2155 (2001)). Even the rotameric states of the

interfacial residues in  $\gamma$ c and gp130 remain largely unchanged upon binding to different cytokines (Wang et al., *Annual review of immunology* **27**: 29-60 (2009)).  $\gamma$ c and gp130 use “chemically inert complementary surfaces”, with a shared hydrophobic core region surrounded by peripheral polar patches, to recognize short-chain and long-chain cytokines, respectively (Boulanger et al., *Molecular cell* **12**: 577-589 (2003a); Wang et al., *Annual review of immunology* **27**: 29-60 (2009)). The recently determined type I IFN receptor complexes revealed that paralogs IFN $\alpha$ 2 and IFN $\omega$  are sandwiched between the first three FNIII domains of IFNAR1 (SD1-SD3) and the two FNIII-like domains of the more compact IFNAR2 (D1, D2) (Thomas et al., *Cell* **146**: 621-632 (2011)). Interestingly, although the N-terminal SD1 domain rotates relative to the SD2-SD3 segment of IFNAR1 upon IFN-binding, once bound to the IFN, IFNAR1 exhibits nearly identical conformations regardless of the identity of the bound cytokine. Both IFNAR1 and IFNAR2 rely on a few conserved “anchor point” residues on the surface of type I IFNs for cross-reactivity (Thomas et al., *Cell* **146**: 621-632 (2011)). However a number of less conserved amino acids are interspersed across the constellation of the conserved IFNs binding surface for fine-tuning their individual binding affinity towards the receptor.

By comparison, the structural basis of CSF-1R promiscuity is quite different from the aforementioned shared cytokine receptors. The core and peripheral binding interface architecture on CSF-1R is somewhat similar to the class I cytokine/receptor recognition paradigm, although CSF-1R uses mainly polar interactions in Site 1 as its core, instead of hydrophobic interactions as observed in  $\gamma$ c and gp130. CSF-1R clearly relies to a greater extent on its conformational plasticity to enable cross-reactivity, a structural adaptation not seen in other shared cytokine receptors. This is perhaps not surprising, considering IL-34 shares merely 11% sequence identity with CSF-1 and has escaped the recognition by predictive bioinformatics routines (Conklin et al., *Bioinformatics* **21**: 1776-1781 (2005)), though more sensitive fold recognition methods fare better (JFB, unpublished). CSF-1R manages to keep impressive affinity towards IL-34 and CSF-1 during evolution by a combination of inter-domain structural plasticity and compositional changes of interfacial residues. This provides a very efficient though not the only mechanism, as witnessed in other cytokine systems, of degenerate recognition of distantly-related helical cytokines.

## **Example 2: Characterization of human anti-IL-34 antibodies**

### **Methods**

#### **Epitope mapping by competition ELISA**

ELISA plates were coated with 1H muIL-34flag (1 ug/ml) in PBS at 4°C overnight or at room temperature for 2 hours and blocked using PBS with 1%BSA and 0.15% Tween20.

Biotinylated YW404.33 antibody at a concentration of 30 nmolar was added to seven serial dilutions of anti-IL-34 antibody to be tested starting at a concentration of 300 nmolar. The antibody mixtures were briefly preincubated at room temperature. The mixtures were then added to the coated ELISA plates and incubated for about 30 minutes to about 1 hour at room temperature. Plates were washed and bound biotinylated antibody was detected with SA-HRP. Anti-muIL-34 and Herceptin were used as controls in this assay.

#### Results

Human anti-IL-34 antibodies were generated using phage display technology and tested for their ability to neutralize IL-34 activity in cell based assays as described in Example 1. The neutralizing activity of anti IL-34 antibody YW404.1, YW404.6 and YW404.33, for example, is shown in Figure 9A. The specificity of these antibodies for human versus murine IL-34 as well as their blocking activity, and binding affinity were compared (Table 7). Furthermore, these antibodies were tested in a competition ELISA to determine if they bind to overlapping epitopes on IL-34 (Table 7).

Table 7: Comparison of YW404.1, YW404.6, YW404.33

	<b>h/m Specificity</b>	<b>Blocking Activity</b>	<b>Binding Affinity</b>	<b>Epitope</b>
YW404.1	cross	++	+++	distinct
YW404.6	murine	++	+	overlapped with YW404.33
YW404.33	cross	+++	+++	overlapped with YW404.6

Several human anti-IL-34 antibodies were then affinity-matured using soft randomization strategy and their affinity was measured as described in Example 1. Affinity-matured antibodies were further tested in cell-based neutralization assays as described in Example 1. As shown in Figure 9B, affinity improvement correlated with better cell blocking activity for anti IL-34 Abs YW404.33, YW404.33.12 and YW404.33.56.

Furthermore, IC50 values were determined based on the ability of the antibody to neutralize the bioactivity of IL-34 on mononuclear cells MNFS60. To measure the ability of the antibody to neutralize the bioactivity of flag-tagged mIL-34 on MNFS50, a cell proliferation assay by CellTiter-Glo® was used. Based on cell response to serial dilutions of IL-34, 50 ng/ml of IL-34 amount was selected for determining the antibody neutralizing activity. The

Half Maximal Inhibitory Concentration (IC<sub>50</sub>) is defined as the concentration of antibody required to yield half maximal inhibition of IL-34 activity on cells, when IL-34 is present at a concentration to elicit 70-80% proliferation response.

50 ng/ml hIL-34 was combined with serial dilutions of anti-IL34 mAbs before adding onto cells in a total volume of 100 ul. The antibody inhibition activity was obtained by measuring RLU after incubating the plates at 37 °C for 72 hours. IC<sub>50</sub> was calculated with KaleidaGraph. For example, anti IL-34 Abs YW404.33.56, 404.33 and YW 404.33.93 had IC<sub>50</sub> values of 20.21 ng/ml, 77.42 ng/ml and 31.62 ng/ml, respectively.

The sequences of the variable heavy and light chain and the CDR regions of these antibodies as well as YW404.1, YW404.6, YW405.3, YW404.33.10 YW404.33.12 and YW404.33.11 are shown in Figures 10A and B.

### **Example 3: Inhibition of DSS-induced inflammatory bowel disease in mice using a combination of anti-CSF-1 antibody and anti-IL-34 antibody**

#### **Methods**

Balb/c mice were pre-weighed and randomized prior to treatment and treated as follows. Group 1 (n=8 per group) received normal drinking water without dextran sulfate sodium (DSS) throughout the experiment. Groups 2 – 6 were kept on drinking water with 3% DSS (dextran sulfidum sulfate 3g/100 ml (3%)) from day 0 to day 6 of the experiment. After day 6, groups 2 – 6 received normal drinking water without DSS until sacrificed. Group 2 was treated with 400 ug anti-ragweed antibody (a-RW-mIgG2 a) every other day starting one day before day 0 until day 8 as negative control. Group 3 was treated daily, starting one day before day 0 until day 8, with 25 mg/kg cyclosporine A in 0.9% sodium chloride (CSA), intraperitoneally. Group 4 was treated with 200 ug of an anti-CSF-1 antibody (rat antibody of ATCC #CRL-2702, clone 5A1) and 200 ug a-RW antibody every other day starting one day before day 0 until day 8. Group 5 was treated with 200 ug anti-IL-34 antibody (YW 404.33.12) and 200 ug a-RW antibody every other day starting one day before day 0 until day 8. Group 6 was treated with 200 ug anti-CSF-1 antibody rat antibody of ATCC #CRL-2702, clone 5A1) and 200 ug anti-IL-34 antibody (YW 404.33.12) every other day starting one day before day 0 until day 8. All mice were sacrificed on day 8 and their colitis severity score was determined based on parameters such as cryptloss and infiltration of inflammatory cells including T-cells, B-cells and macrophages.

## Results

Mice with DSS-induced IBD that were treated with either the anti-IL-34 antibody or the anti-CSF-1 antibody showed a reduced colitis severity score as compared to mice treated with the control a-RW antibody. The inhibition of both, CSF-1 and IL-34 using a combination of the anti-CSF-1 antibody and the anti-IL-34 antibody was even more effective in this IBD model (Figure 11).

The involvement of IL-34 and CSF-1 in IBD was further supported by measuring serum levels of IL-34 and CSF-1 in control (no DSS) mice and mice with DSS-induced IBD treated with control antibody (a-RW). The levels of CSF-1 and IL-34 were determined using ELISAs. Mice with DSS-induced IBD treated with control antibody showed elevated IL-34 and CSF-1 levels compared to control mice (Figure 12).

### Example 4 – IL-34 and CSF-1 in human RA patients

Expression of CSF-1, IL-34 and TNF $\alpha$  protein in the serum, synovial fluid and tissue from rheumatoid arthritis patients is shown in Figure 13. IL-34 is expressed at lower levels than CSF-1 and TNF $\alpha$  in those fluids. IHC staining of synovium tissue from RA patients with anti-IL34 antibody indicates that IL-34 is likely enriched in the tissue/extracellular matrix (ECM).

Microarray results measuring gene expression of Myeloid subtype genes in the joints of RA patients who (A) responded to TNF blockade (TNF-R) or did not respond to a TNF blockade (TNF-NR) or (B) who were treated with rituximab after non-responsive treatment with a TNF blockade are shown in Figure 14. Rituximab non-responders are “Rituximab-NR” and rituximab responders are “Rituximab-R.” The Myeloid subtype enriches for anti-TNF responders (TNF-R). The results also indicate that the CSF1/IL34 pathway is involved in primary and secondary TNF-NR RA patients. As the transcripts of IL34 and CSF1 are present at high levels in TNF-NR and Rituximab-NR it is likely that the IL34/CSF1 pathway significantly contributes to pathogenicity in these patients not responding to anti-TNF $\alpha$  or Rituximab therapy. Blocking both cytokines in these patients is likely to be required for clinical benefit.

### Example 5 – Inhibition of both IL-34 and CSF-1 in CIA models

The treatment combination of anti-CSF1 antibody with anti-IL34 antibody was compared against TNFR2-Fc in a mouse CIA model (Figure 15). DBA-1J mice were injected with

bovine collagen type II in CFA at day 0 and 21 by i.d. The arthritis score was then monitored daily. The antibody treatment started at either day 24 or day 31 (arthritis established, clinical score = 4) for 7 weeks before termination. The antibody treatments were: a-ragweed (antibody mIgG2a isotype control), TNFRII-Ig (mIgG2a), anti-muCSF1 (in-house, mIgG2a), anti-IL34 (YW404.33.12, mIgG2a), anti-mCSF1+anti-IL34 (combination of 3 &4), anti-mCSF1 (DANA)+anti-IL34 (YW404.33.12 DANA), All animals (n=10/group) were treated with 200ug/animal, 3 times/week of the above reagents by ip. for 7 weeks before termination. The endpoint PD including longitudinal clinical score, histopathology (paw and related tissues), FACS (tissue monocytes subsets and Mf) and bone volume (uCT) analysis.

The study shows the comparable or trending better inhibition of clinical scores and histology scores (inflammation, fibroplasia, cartilage) with the combination of anti-CSF1 antibody and anti-IL-34 antibody over TNFRII-Fc, especially with the antibodies that have reduced ADCC activity (i.e., DANA mutation is D265A, N297A in the Fc region). Furthermore, the treatment with a combination of aCSF1+aIL34 is clearly superior compared to TNFRII-Fc in protecting against bone erosions, and furthermore, treatment with the combination of anti-CSF1 and anti-IL-34 antibodies is superior to either one alone.

#### **Example 6 – Inhibition of both IL-34 and CSF-1 in additional IBD models**

In another model for IBD, DSS colitis was induced in C57BL/6J Female, 6-8 wk old mice (Figure 16). C57B6 mice were orally administered 3% DSS for 5 days to induce acute colitis, characterized by epithelial damage, reversible weight loss and neutrophilic infiltration in large intestine. The antibody treatment started at -1 days with 4 doses total. The study was terminated at day 8 for analysis. The treatment groups (n=10/group) : 3% DSS + a-ragweed (mIgG2a control, 400 ug/mouse. ip), 3% DSS + a-mCSF1 Ab (in house, 200ug/mouse, ip), 3% DSS + a-IL34 Ab (YW404.33.12, 200ug/mouse ip.), 3% DSS + a-mCSF1/a-IL34 (200ug/mouse a-CSF1 and 200ug/mouse a-IL34, ip.). Dexamethasone was administered at 0.5 mg/kg, QD, ip. The naïve mice were not treated with DSS. Anti-ragweed antibody was used as a negative control. After termination the colon histology score was readout for this model.

Analysis of TNFΔARE colitis mice showed that there is higher CSF-1/IL-34 and CSF-1R protein and mRNA expression when there is higher myeloid cell proliferation and activation (data not shown). Mf and monocytes were significantly proliferated and activated in TNFΔARE mice spleen. TNFΔARE mice produced higher CSF-1 and IL-34 in gut tissue

compared to wild-type (wt) mice. Higher CSF-1R, CSF1 & IL-34 mRNA expression levels were observed in TNFΔARE mice ileum compared to wild-type mice.

#### **Example 7 – IL-34 and CSF-1 in human Crohn's and UC patients**

An analysis of CSF-1 and IL-34 protein from serum and tissue of human patients suffering from Crohn's disease or ulcerative colitis showed that IL-34 is expressed at very low levels in the sera, but is expressed at higher levels in tissue. CSF-1, on the other hand, is expressed well in the sera and in tissue (Figure 17). This tissue data further supports the belief that inhibition of both IL-34 and CSF1 in inflammatory bowel disease, including ulcerative colitis and Crohn's disease, will be superior to treating IBD patients with inhibitors of either alone.

In RA patients, IL-34 protein expression was lower than CSF1, but easily detectable in serum and synovial fluid. In those same patients, CSF-1 protein expression was highly expressed in both the serum and the synovial fluid (Figure 13).

An analysis of IL-34, CSF-1 and TNFα protein in the synovial fluid of RA and osteoarthritis patients showed that there is no correlation between expression levels of IL-34/CSF-1 and TNFα in those fluids in rheumatoid arthritis and osteoarthritis patients (Figure 18). Therefore, the data indicates that are patients suffering from RA and osteoarthritis that have distinct molecular profiles, i.e., TNFα expression versus CSF-1/IL-34 expression, despite their similar phenotypes.

#### **Example 8 – Inhibition of IL-34 and CSF-1 reduces the infiltration of myeloid cells**

Mouse CIA was set up as mentioned previously. The animal joint synovial cell/tissue were collected from the mice treated by a-CSF1+a-IL34 combination Abs or anti-ragweed for twice at beginning on day 39 (established CIA, clinical score = 4) in 7 days period (n=3 / sample). Animal were euthanized after 7 days treatment. The joint synovial tissue/cells were collected and collagenase digested for single cell suspension. Cells were stained with labeled anti-CD11b, Ly6C, Ly6G and F4/80 antibodies (purchase from BD Biosystem). Labeled cells were analyzed by flow cytometer to defined the myeloid subsets (Mf: CD11b+/F480+, inflammatory monocytes: CD11b+/Ly6C+/Ly6Glow and residential monocytes: CD11b+/Ly6G+/Ly6Clow).

This data shows the reduction of mouse myeloid cells (Mf and monocytes) infiltrating joint synovia after only 7 days of anti-CSF1/IL-34 combination treatment of already arthritic animals before any clinical benefit can be observed. This shows that a major mechanism for

myeloid inhibition as the result of combo therapy inhibition is the reduction of myeloid cell migration, infiltration and or expansion in the synovium.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be

5 construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference. These

disclosures include the publication cited as Ma et al., (2012) Structure 20:676-687 and United States Provisional Application No. 61,595,658, filed February 6, 2012 and United States Provisional Application No. 61/680,674, filed August 7, 2012, from which this

10 application claims benefit, all of which are incorporated by reference in their entirety.



## CLAIMS

## WHAT IS CLAIMED IS:

1. An isolated antibody that binds to human IL-34, which antibody binds to an epitope comprising at least one of amino acid residues Glu103, Leu109, Gln106, Asn150, Leu127, Asn128, Ser184, Leu186, Asn187, Lys44, Glu121, Asp107, Glu111, Ser104, Gln120, Trp116, and Asn61 of a human IL-34, wherein the position of the amino acid residues is based on the position in SEQ ID NO:1, and wherein the antibody inhibits the binding between human IL-34 and human CSF-1R.
2. An isolated antibody that binds to human IL-34, which antibody binds to an epitope comprising at least one of amino acid residues from Glu103 to Asn150 of a human IL-34, wherein the position of the amino acid residues is based on SEQ ID NO:1, and wherein the antibody inhibits the binding between human IL-34 and human CSF-1R.
3. The antibody of claim 1 or claim 2, which antibody binds to an epitope comprising at least one of amino acid residues Glu103, Leu109, Gln106, and Asn150 of the human IL-34, wherein the position of the amino acid residues is based on the position in SEQ ID NO:1.
4. The antibody of claim 3, wherein the epitope further comprises at least one of amino acid residues Ser100, Glu123, Trp116, Thr124, Leu127, Asn128, Gln131, and Thr134 of the human IL-34, wherein the position of the amino acid residues is based on the position in SEQ ID NO:1.
5. The antibody of claim 3 or claim 4, wherein the antibody binds to amino acids within positions 100-108, 116-134, 109 and 150 of the human IL-34, and wherein the position of the amino acid residues is based on the position in SEQ ID NO:1.
6. The antibody of claim 1, which antibody binds to an epitope comprising at least one of amino acid residues Asn128, Ser184, Leu186, Asn187, Lys44, and Glu121 of the human IL-34, wherein the position of the amino acid residues is based on the position in SEQ ID NO:1.
7. The antibody of claim 6, wherein the epitope further comprises at least one of amino acid residues Phe40, Asp43, Leu125, Gln189, Thr36, and Val185 of the human IL-34, wherein the position of the amino acid residues is based on the position in SEQ ID NO:1.

8. The antibody of claim 6 or claim 7, wherein the antibody binds to amino acids within positions 36-44, 121-128, and 184-187 of the human IL-34, and wherein the position of the amino acid residues is based on the position in SEQ ID NO:1.

9. The antibody of claim 1 or claim 2, which antibody binds to an epitope comprising at least one of amino acid residues from Glu103-Leu127 of the human IL-34, wherein the position of the amino acid residues is based on the position in SEQ ID NO:1.

10. The antibody of claim 1, which antibody binds to an epitope comprising at least one of amino acid residues Asp107, Glu111, Ser104, Gln120, Glu103, Leu109, Trp116, and Asn61 of the human IL-34, wherein the position of the amino acid residues is based on the position in SEQ ID NO:1.

11. The antibody of claim 10, wherein the epitope further comprises at least one of amino acid residues Pro152, Val108, Leu110, Gln106, Glu123, Leu127, Lys117, Ile60 and Lys55 of the human IL-34, wherein the position of the amino acid residues is based on the position in SEQ ID NO:1.

12. The antibody of claim 10 or claim 11, wherein the antibody binds to amino acids within positions 55-61, 100-108, 109, 111-127 and 152 of the human IL-34, and wherein the position of the amino acid residues is based on the position in SEQ ID NO:1.

13. The antibody of any one of claims 10 to 12, wherein the antibody comprises a heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:3 and/or a light chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:4.

14. The antibody of any one of claims 10 to 13, wherein the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:3 and/or a light chain variable region sequence of the amino acid sequence of SEQ ID NO:4..

15. The antibody of any one of claims 10 to 12, wherein the antibody comprises (a) a HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); (b) a HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39); and (c) a HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52).

16. The antibody of any one of claims 10 to 12, wherein the antibody comprises (a) a HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59); (b) a HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52); and (c) a HVR-H3 comprising an amino acid sequence  
5 GLGKGSKRGAMDY (SEQ ID NO: 33).

17. The antibody of any one of claims 10 to 12 and 16, wherein the antibody comprises (a) a HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (b) a HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) a HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ  
10 ID NO: 39).

18. The antibody of any one of claims 1-17, wherein the antibody binds to a dimer of the IL-34.

19. The antibody of claim 18, wherein the antibody binds to an epitope that spans over both protomers of the human IL-34 dimer.

20. An isolated antibody that binds to a human IL-34, wherein the antibody inhibits the binding between human IL-34 and human CSF-1R, and wherein the antibody binds to a dimer of the IL-34.

21. The antibody of claim 20, wherein the antibody comprises (a) a HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33) or  
20 GINQGSKRGAMDY (SEQ ID NO: 32); (b) a HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39) or QQSYTTPPT (SEQ ID NO: 43) or QQYTALPYT (SEQ ID NO: 49) or QQYSDLPYT (SEQ ID NO: 45) or QQYSDVPYT (SEQ ID NO: 47) or QQSRTARPT (SEQ ID NO: 41); and (c) a HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52) or RISPYSGYTNYADSVKG (SEQ ID NO: 51).  
25

22. The antibody of claim 20, wherein the antibody comprises (a) a HVR-H1 comprising an amino acid sequence STWIIH (SEQ ID NO: 59); (b) a HVR-H2 comprising an amino acid sequence RISPYYYYSDYADSVKG (SEQ ID NO: 52) or RISPYSGYTNYADSVKG (SEQ ID NO: 51); and (c) a HVR-H3 comprising an  
30 amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33) or GINQGSKRGAMDY (SEQ ID NO: 32).

23. The antibody of claim 20 or claim 22, wherein the antibody comprises (a) a HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50);

(b) a HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and  
(c) a HVR-L3 comprising an amino acid sequence QQSFYFPNT (SEQ ID NO: 39) or  
QQSYTTPPT (SEQ ID NO: 43) or QQYTALPYT (SEQ ID NO: 49) or  
QQYSDLPYT (SEQ ID NO: 45) or QQYSDVPYT (SEQ ID NO: 47) or  
5 QQSRTARPT (SEQ ID NO: 41) or QQSFYFPN (SEQ ID NO: 38) or QQSYYTTPP  
(SEQ ID NO: 42) or QQYTALPY (SEQ ID NO: 48) or QQYSDLPY (SEQ ID NO:  
44) or QQYSDVPY (SEQ ID NO: 46) or QQSRTARP (SEQ ID NO: 40).

24. The antibody of claim 20, wherein the antibody comprises (a) a HVR-H3  
comprising an amino acid sequence GLGKGSKRGAMDY (SEQ ID NO: 33); (b) a  
10 HVR-L3 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45); and  
(c) a HVR-H2 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ  
ID NO: 51).

25. The antibody of claim 20, wherein the antibody comprises (a) a HVR-H1  
comprising an amino acid sequence of STWIIH (SEQ ID NO: 59); (b) a HVR-H2  
15 comprising an amino acid sequence RISPYSGYTNYADSVKG (SEQ ID NO: 51);  
and (c) a HVR-H3 comprising an amino acid sequence GLGKGSKRGAMDY (SEQ  
ID NO: 33).

26. The antibody of claim 20 or claim 25, wherein the antibody comprises (a) a  
HVR-L1 comprising an amino acid sequence of RASQDVSTAVA (SEQ ID NO: 50);  
20 (b) a HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and  
(c) a HVR-L3 comprising an amino acid sequence QQYSDLPYT (SEQ ID NO: 45).

27. The antibody of any one of claims 20 to 23, wherein the antibody comprises a  
heavy chain variable region sequence of at least 90% sequence identity to the amino  
acid sequence of SEQ ID NO:5 and/or a light chain variable region sequence of at  
25 least 90% sequence identity to the amino acid sequence of SEQ ID NO:6.

28. The antibody of any one of claims 20 to 23, wherein the antibody comprises a  
heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:5  
and/or a light chain variable region sequence of the amino acid sequence of SEQ ID  
NO:6.

29. The antibody of any one of claims 20 to 23, wherein the antibody comprises a  
heavy chain variable region sequence of at least 90% sequence identity to the amino  
acid sequence of SEQ ID NO:7 and/or a light chain variable region sequence of at  
30 least 90% sequence identity to the amino acid sequence of SEQ ID NO:8.

30. The antibody of any one of claims 20 to 23, wherein the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:7 and/or a light chain variable region sequence of the amino acid sequence of SEQ ID NO:8.

31. The antibody of any one of claims 20 to 23, wherein the antibody comprises a heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:9 and/or a light chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:10.

32. The antibody of any one of claims 20 to 23, wherein the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:9 and/or a light chain variable region sequence of the amino acid sequence of SEQ ID NO:10.

33. The antibody of any one of claims 20 to 26, wherein the antibody comprises a heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11 and/or a light chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:12.

34. The antibody of any one of claims 20 to 26, wherein the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:11 and/or a light chain variable region sequence of the amino acid sequence of SEQ ID NO:12.

35. The antibody of any one of claims 20 to 23, wherein the antibody comprises a heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:13 and/or a light chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:14.

36. The antibody of any one of claims 20 to 23, wherein the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:13 and/or a light chain variable region sequence of the amino acid sequence of SEQ ID NO:14.

37. The antibody of any one of claims 20-36, wherein the antibody binds to an epitope that spans over both protomers of the human IL-34 dimer.

38. The antibody of any one of claims 20-37, wherein the antibody neutralizes IL-34 activity.

39. An isolated antibody that binds to human IL-34, wherein the antibody inhibits the binding between human IL-34 and human CSF-1R, and wherein the antibody neutralizes IL-34 activity.

40. The antibody of claim 39, wherein the antibody comprises (a) a HVR-H3 comprising an amino acid sequence SRGAYRFAY (SEQ ID NO: 56); (b) a HVR-L3 comprising an amino acid sequence QQSYTTPPT (SEQ ID NO: 43); and (c) a HVR-H2 comprising an amino acid sequence SITPASGDDYADSVKG (SEQ ID NO: 54).

41. The antibody of claim 39, wherein the antibody comprises (a) a HVR-H1 comprising an amino acid sequence SNYIH (SEQ ID NO: 55); (b) a HVR-H2 comprising an amino acid sequence SITPASGDDYADSVKG (SEQ ID NO: 54); and (c) a HVR-H3 comprising an amino acid sequence SRGAYRFAY (SEQ ID NO: 56).

42. The antibody of claim 39 or claim 41, wherein the antibody comprises (a) a HVR-L1 comprising an amino acid sequence RASQDVSTAVA (SEQ ID NO: 50); (b) a HVR-L2 comprising an amino acid sequence SASFLYS (SEQ ID NO: 53); and (c) a HVR-L3 comprising an amino acid sequence QQSYTTPPT (SEQ ID NO: 43).

43. The antibody of any one of claims 39-42, wherein the antibody comprises a heavy chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:15 and/or a light chain variable region sequence of at least 90% sequence identity to the amino acid sequence of SEQ ID NO:16.

44. The antibody of any one of claims 39-43, wherein the antibody comprises a heavy chain variable region sequence of the amino acid sequence of SEQ ID NO:15 and/or a light chain variable region sequence of the amino acid sequence of SEQ ID NO:16.

45. The antibody of any one of the preceding claims, wherein the antibody does not inhibit the binding between human CSF-1 and human CSF-1R.

46. The antibody of any one of the preceding claims, wherein the antibody is a monoclonal antibody.

47. The antibody of any one of the preceding claims, wherein the antibody is a human, humanized or chimeric antibody.

48. The antibody of any of the preceding claims, wherein the antibody is a bispecific antibody.

49. The antibody of claim 48, wherein the bispecific antibody comprises a second binding specificity to human CSF-1.

50. A bispecific antibody comprising a first binding specificity to human IL-34 (SEQ ID NO. 1) and a second binding specificity to human CSF-1.

51. The bispecific antibody of claim 50, wherein the antibody inhibits binding of human IL-34 to human CSF-1R and inhibits binding of human CSF-1 to human CSF-1R.

52. The antibody of any one of the preceding claims, which is an antibody fragment that binds human IL-34.

53. The fragment of claim 52, wherein the fragment is a Fab, Fab', Fab'-SH, F(ab')<sub>2</sub>, Fv or scFv fragment.

54. The antibody of any one of claims 1-51, wherein the antibody is a one-armed antibody.

55. The antibody of any one of claims 1-51, wherein the antibody is a linear antibody.

56. The antibody of any one of the preceding claims, which is a full length IgG1 or an IgG4 antibody.

57. An isolated antibody that binds human CSF-1R, which antibody binds to an epitope comprising at least one of amino acid residues Arg144, Gln248, Gln249, Ser250, Phe252, and Asn254 of human CSF-1R, wherein the position of amino acid residue is based on the position in SEQ ID NO:2, and wherein the antibody inhibits the binding between human IL-34 and human CSF-1R.

58. The antibody of claim 57, wherein the antibody binds to an epitope comprising amino acid residue Arg144 of CSF-1R, wherein the position of amino acid residue is based on the position in SEQ ID NO:2.

59. The antibody of claim 58, wherein the epitope further comprises at least one of amino acid residues Arg142, Arg146, and Arg150 of human CSF-1R, and wherein the position of amino acid residues is based on the position in SEQ ID NO:2.

60. The antibody of claims 58 or 59, wherein the epitope further comprises at least one of amino acid residues Ser172 and Arg192 of human CSF-1R, and wherein the position of amino acid residues is based on the position in SEQ ID NO:2.

61. The antibody of any one of claims 58 to 60, wherein the epitope further comprises at least one of amino acid residues Arg146, Met149, Arg150, Phe169,

Ile170, and Gln173 of human CSF-1R, and wherein the position of amino acid residues is based on the position in SEQ ID NO:2.

62. The antibody of any one of claims 58 to 61, wherein the antibody binds to amino acids within positions 142-150 and 169-173, and wherein the position of amino acid residues is based on the position in SEQ ID NO:2.

63. The antibody of claim 57, wherein the antibody binds to an epitope comprising at least one of amino acid residues Gln248, Gln249, Ser250, Phe252, and Asn254 of human CSF-1R, wherein the position of amino acid residue is based on the position in SEQ ID NO:2.

64. The antibody of claim 63, wherein the epitope further comprises amino acid residue Tyr257 of human CSF-1R, and wherein the position of amino acid residue is based on the position in SEQ ID NO:2.

65. The antibody of claims 63 or 64, wherein the epitope further comprises at least one of amino acid residues Pro247, Gln258, and Lys259 of human CSF-1R, and wherein the position of amino acid residues is based on the position in SEQ ID NO:2.

66. The antibody of any one of claims 63 to 65, wherein the epitope further comprises at least one of amino acid residues Val231, Asp251, and Tyr257 of human CSF-1R, and wherein the position of amino acid residue is based on the position in SEQ ID NO:2.

67. The antibody of any one of claims 63 to 66, wherein the antibody binds to amino acid residues within positions 231, 248-252, and 254, and wherein the position of amino acid residues is based on the position in SEQ ID NO:2.

68. An isolated nucleic acid encoding the antibody of any one of the preceding claims.

69. A vector comprising the nucleic acid of claim 68.

70. A host cell comprising the nucleic acid of claim 69.

71. A method of producing an antibody, comprising culturing the host cells of claim 70 so that the antibody is produced.

72. The method of claim 71, further comprising recovering the antibody produced by the host cell.

73. A pharmaceutical composition comprising the antibody of any one of claims 1-67, and a pharmaceutically acceptable carrier.

74. The antibody of any one of claims 1-67 for use as a medicament.



75. The antibody of any one of claims 1-67 for use in treating a myeloid pathogenic immunological disease.
76. The antibody of any one of claims 1-67 for use in inhibiting binding between human IL-34 and human CSF-1R.
- 5 77. Use of the antibody of any one of claims 1-67 in the manufacture of a medicament.
78. The use of claim 77, wherein the medicament is for treating a myeloid pathogenic immunological disease.
79. The use of claim 77, wherein the medicament inhibits binding between both  
10 human IL-34 and human CSF-1R and human CSF1 and human CSF1R.
80. A method of treating an individual having a myeloid pathogenic immunological disease comprising administering to the individual an effective amount of the antibody of any one of claims 1-67.
81. A method of treating an individual having a myeloid pathogenic  
15 immunological disease comprising administering to the individual an effective amount of the antibody of any one of claims 1-47 in conjunction with an antibody that binds human CSF-1.
82. The method of claim 80, wherein the antibody is a bispecific antibody and wherein the activity of human IL-34 and human CSF-1 is inhibited.
- 20 83. The method of claim 81, wherein the activity of human IL-34 and human CSF-1 is inhibited.
84. The method of claim 82 or claim 83, wherein the binding of human IL-34 to human CSF-1R is inhibited, and wherein the binding of human CSF-1 and human CSF-1R is inhibited.
- 25 85. The method of any one of claims 80 to 84, wherein the myeloid pathogenic immunological disease is rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, macrophage activated syndrome (MAS), discoid lupus, sarcoidosis, vasculitis, and graft versus host disease.
86. A method of inhibiting binding between human IL-34 and human CSF-1R in  
30 an individual comprising administering to the individual an effective amount of the antibody of any one of claims 1-67.

87. A method of any one of claims 80-86, where in the individual to be treated has inadequate response to a TNF therapy and/or rituximab therapy for the myeloid pathogenic disease.

88. An article of manufacture comprising the antibody of any one of claims 1-67.

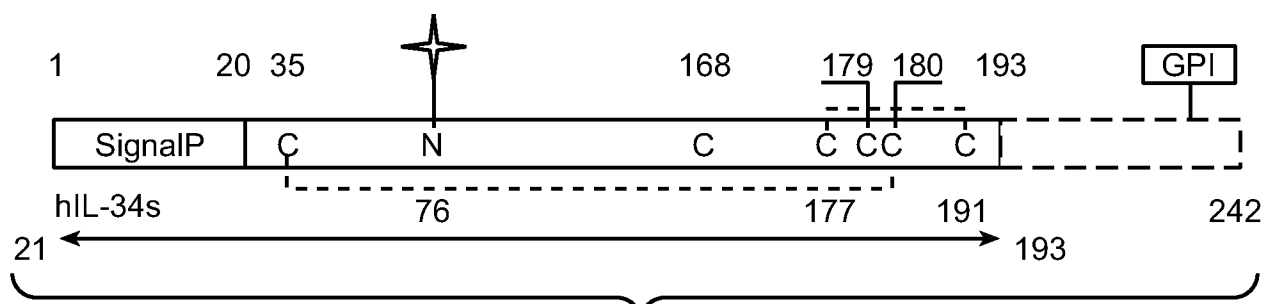
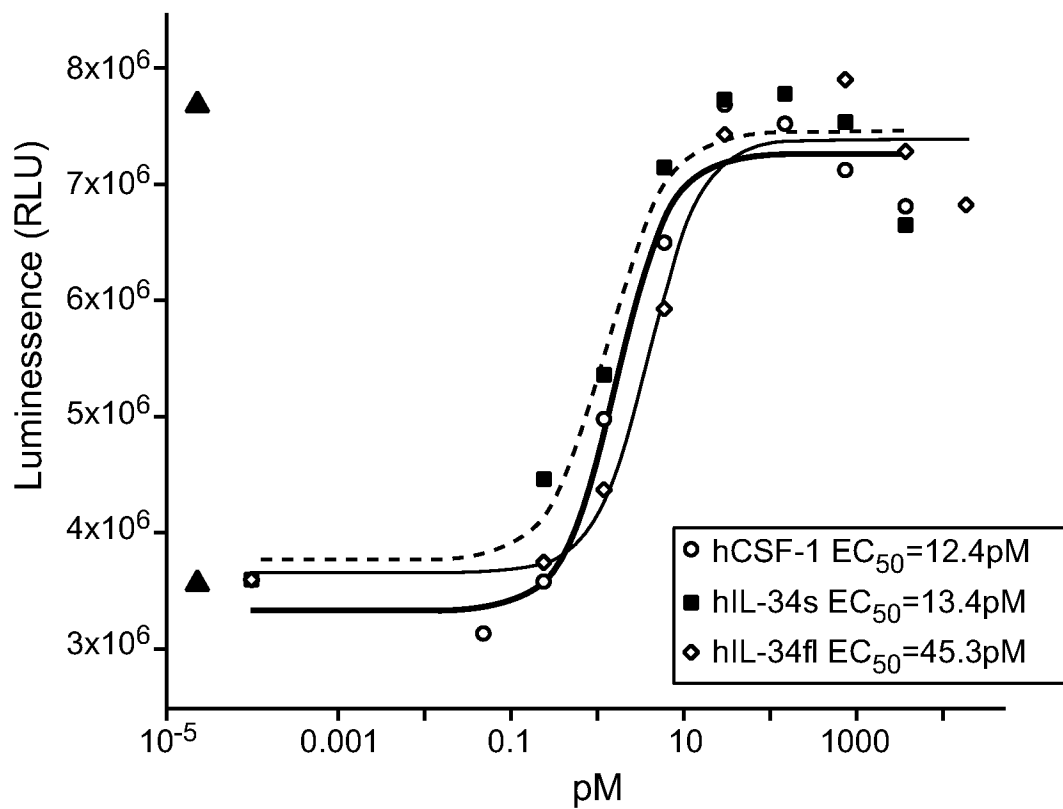
5 89. An article of manufacture comprising the antibody of any one of claims 1-47, and further comprising an antibody that binds to human CSF-1.

90. The article of manufacture of claim 88, further comprising instructions for administering an effective amount of the antibody to an individual for treating a myeloid pathogenic immunological disease in the individual.

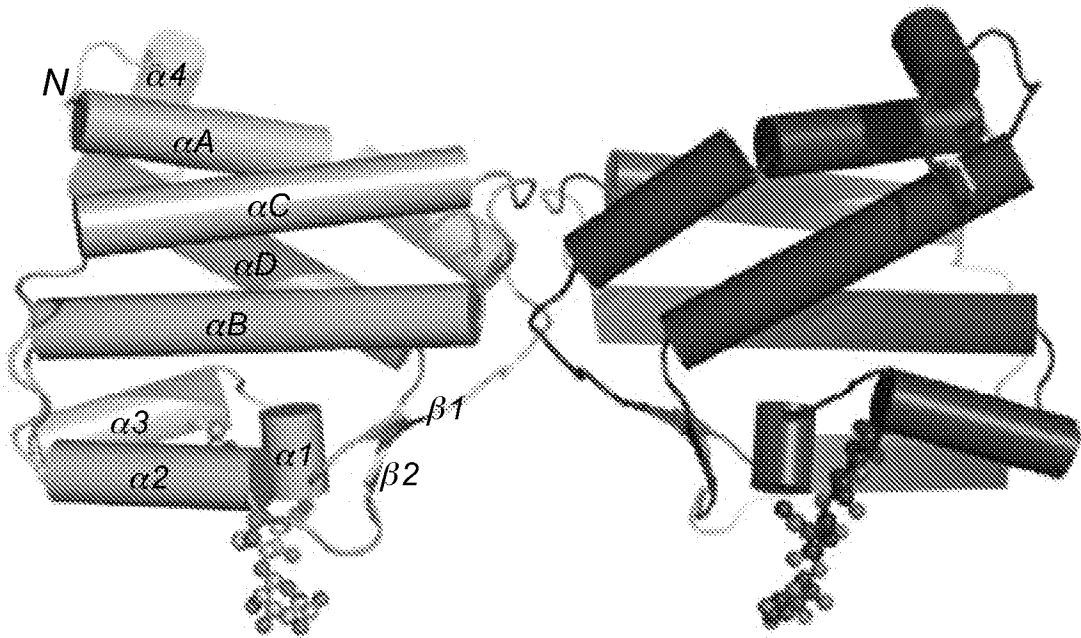
10 91. The article of manufacture of claim 88, further comprising instructions for administering an effective amount of the antibody of any one of claims 1-47 and the antibody that binds to human CSF-1 to an individual for treating a myeloid pathogenic immunological disease in the individual.

15 92. The article of manufacture of any one of claims 87 to 91, wherein the myeloid pathogenic immunological disease is rheumatoid arthritis, inflammatory bowel disease or multiple sclerosis.

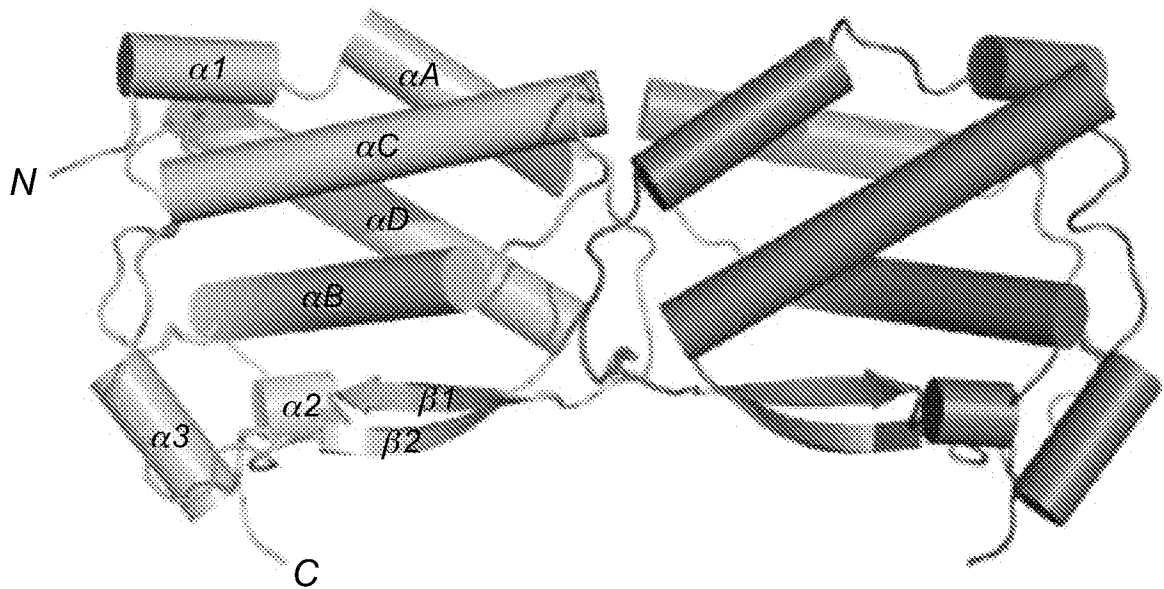
1 / 28

**FIG. 1A****FIG. 1B**

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**FIG. 1C**



**FIG. 1D**

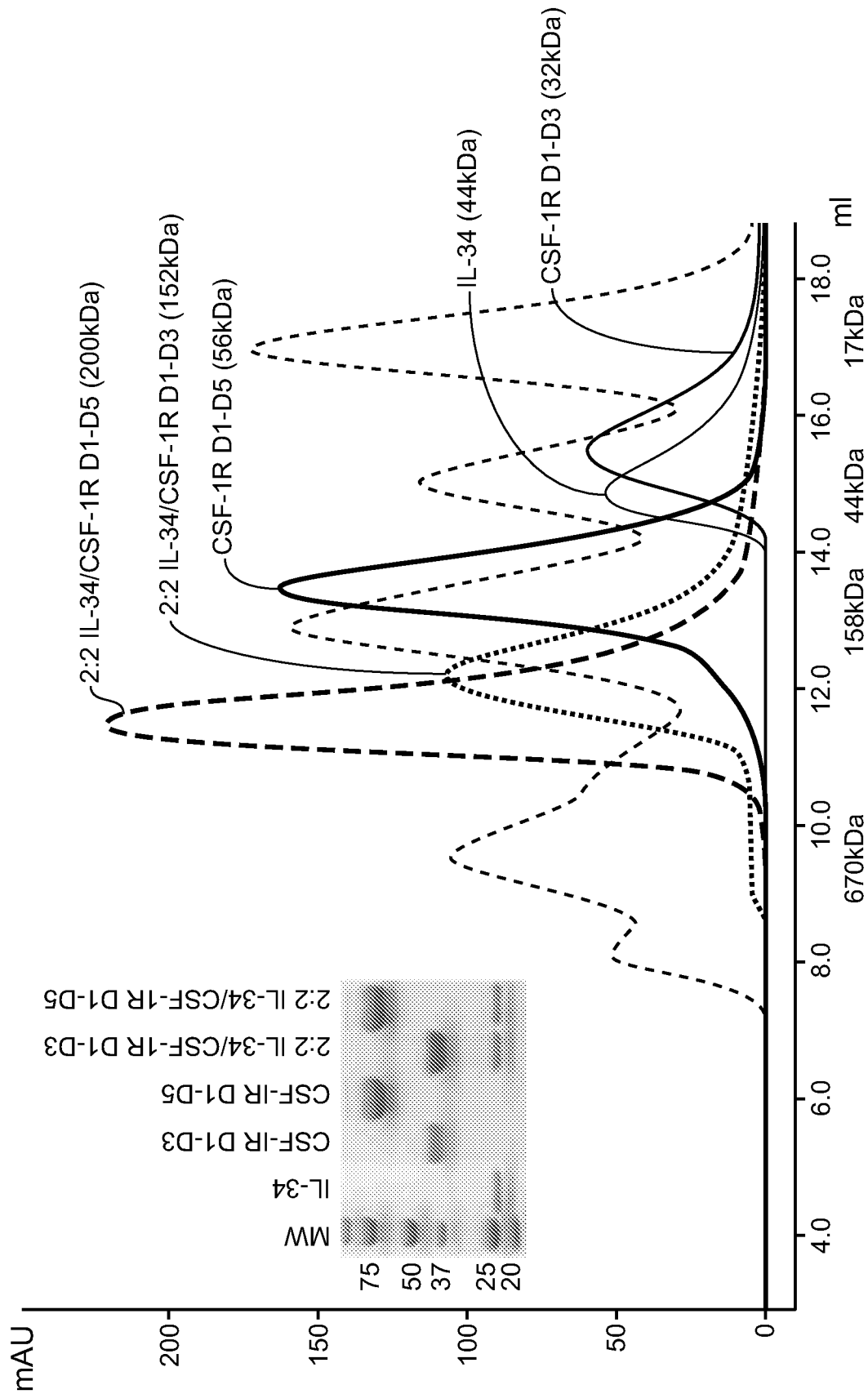
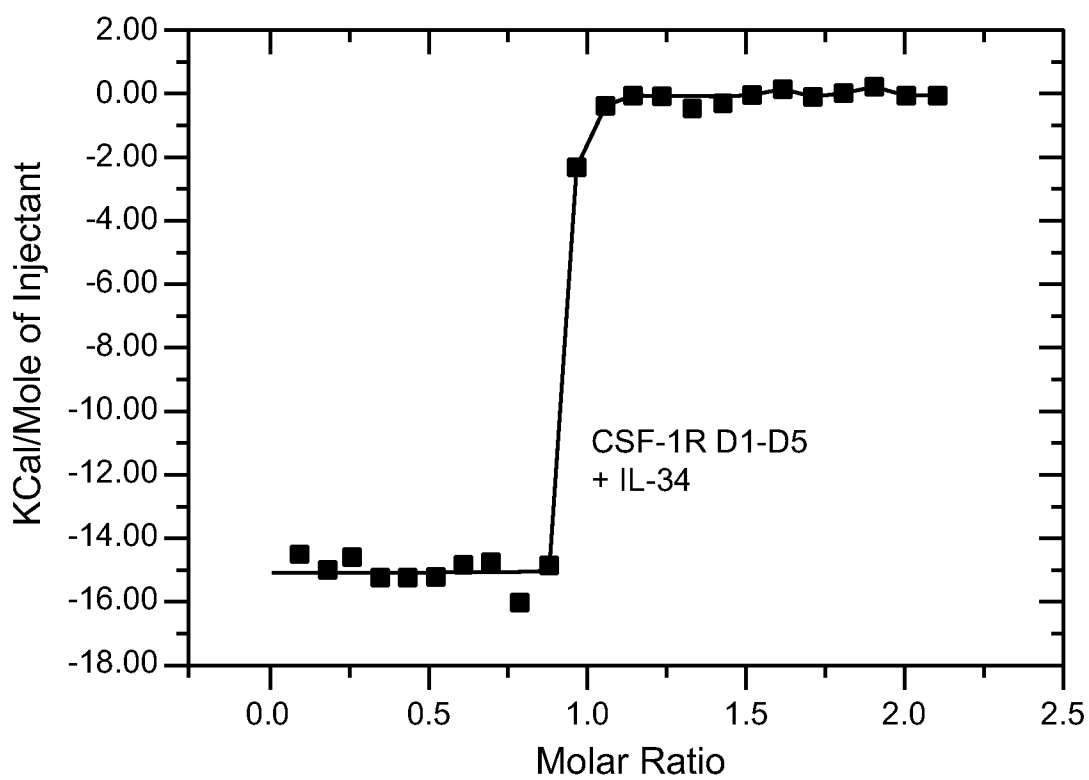
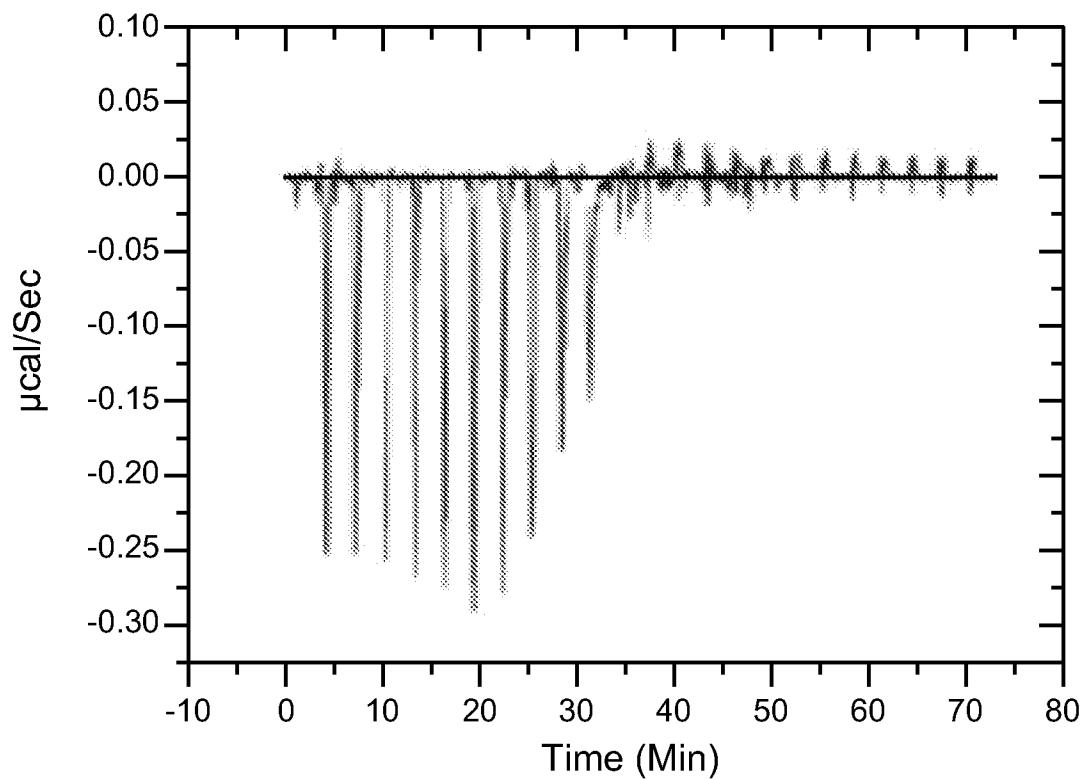
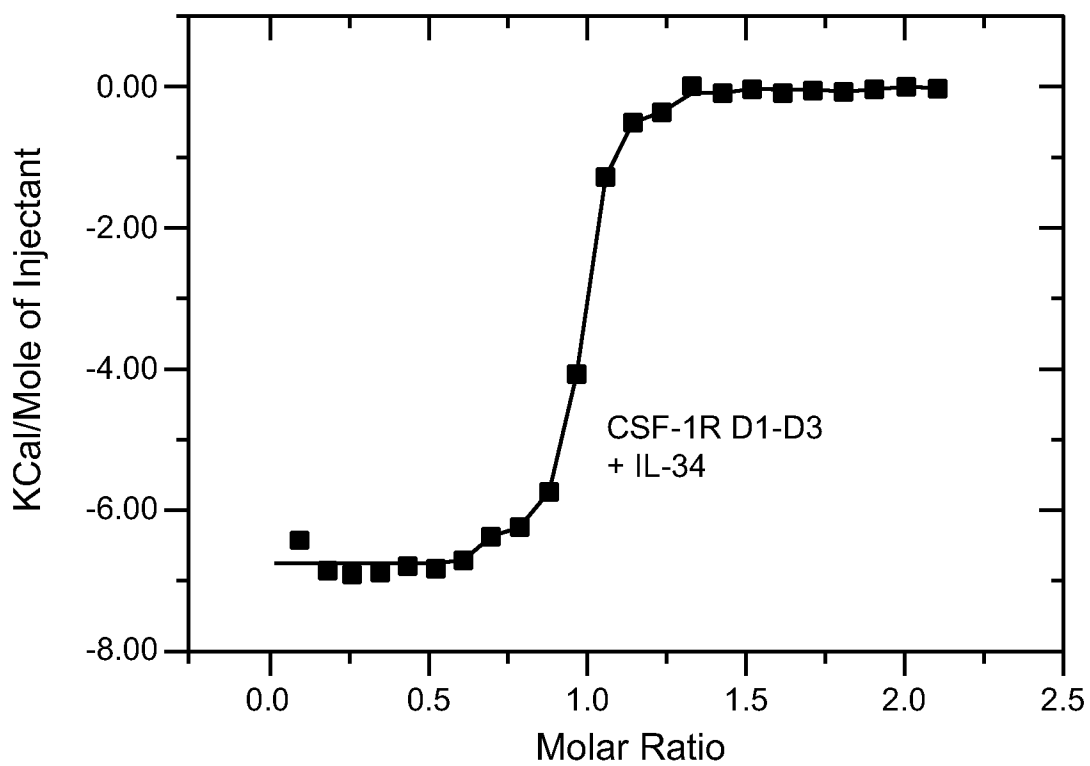
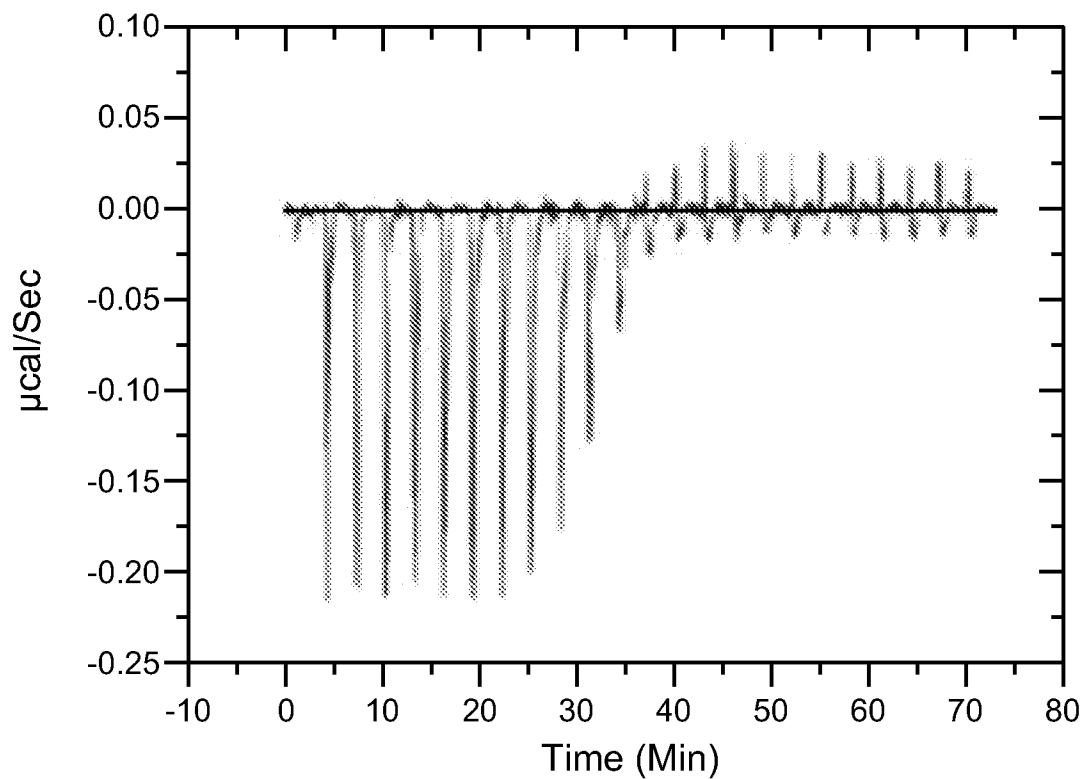


FIG. 2A

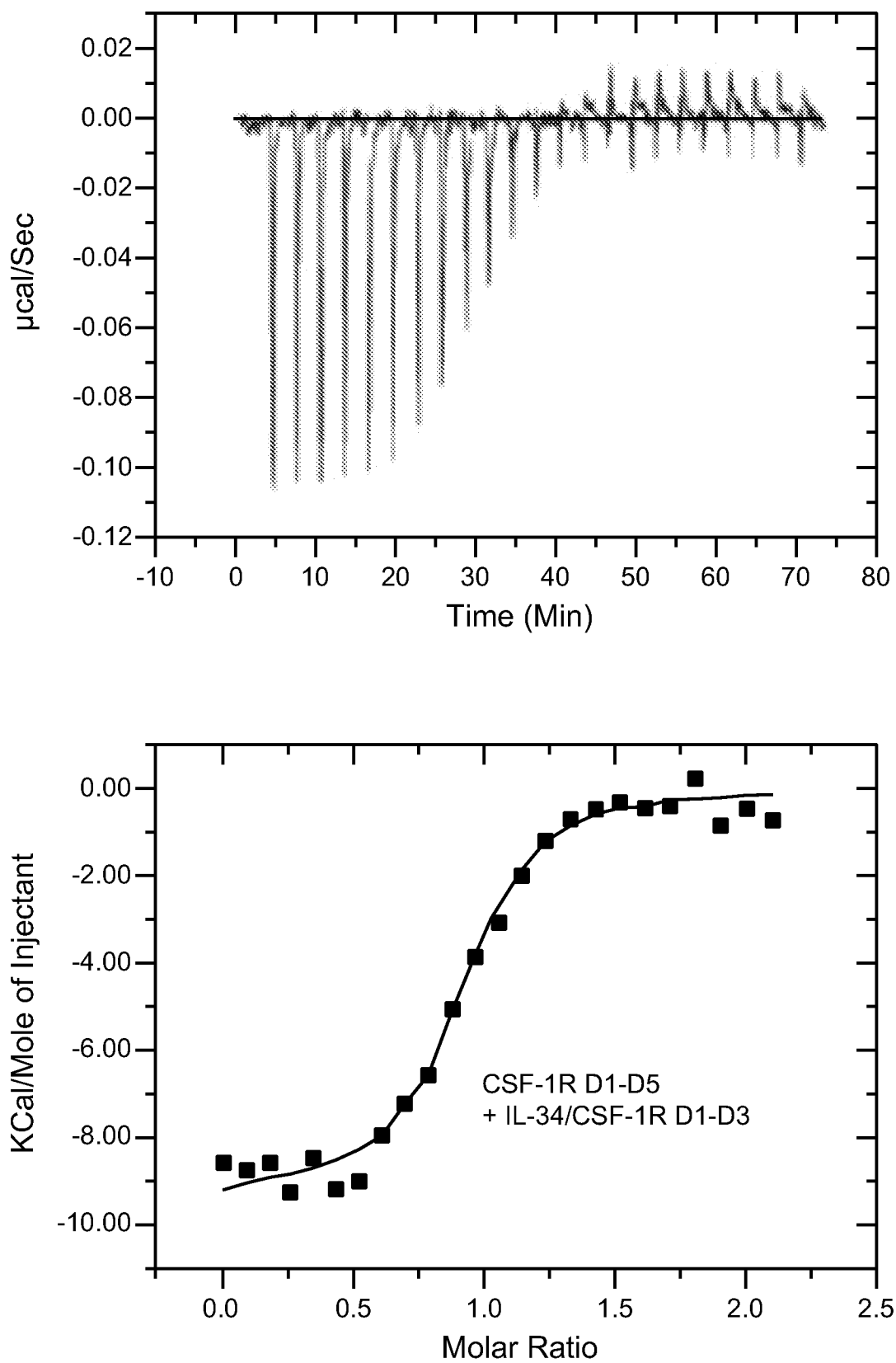
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**FIG. 2B-1**

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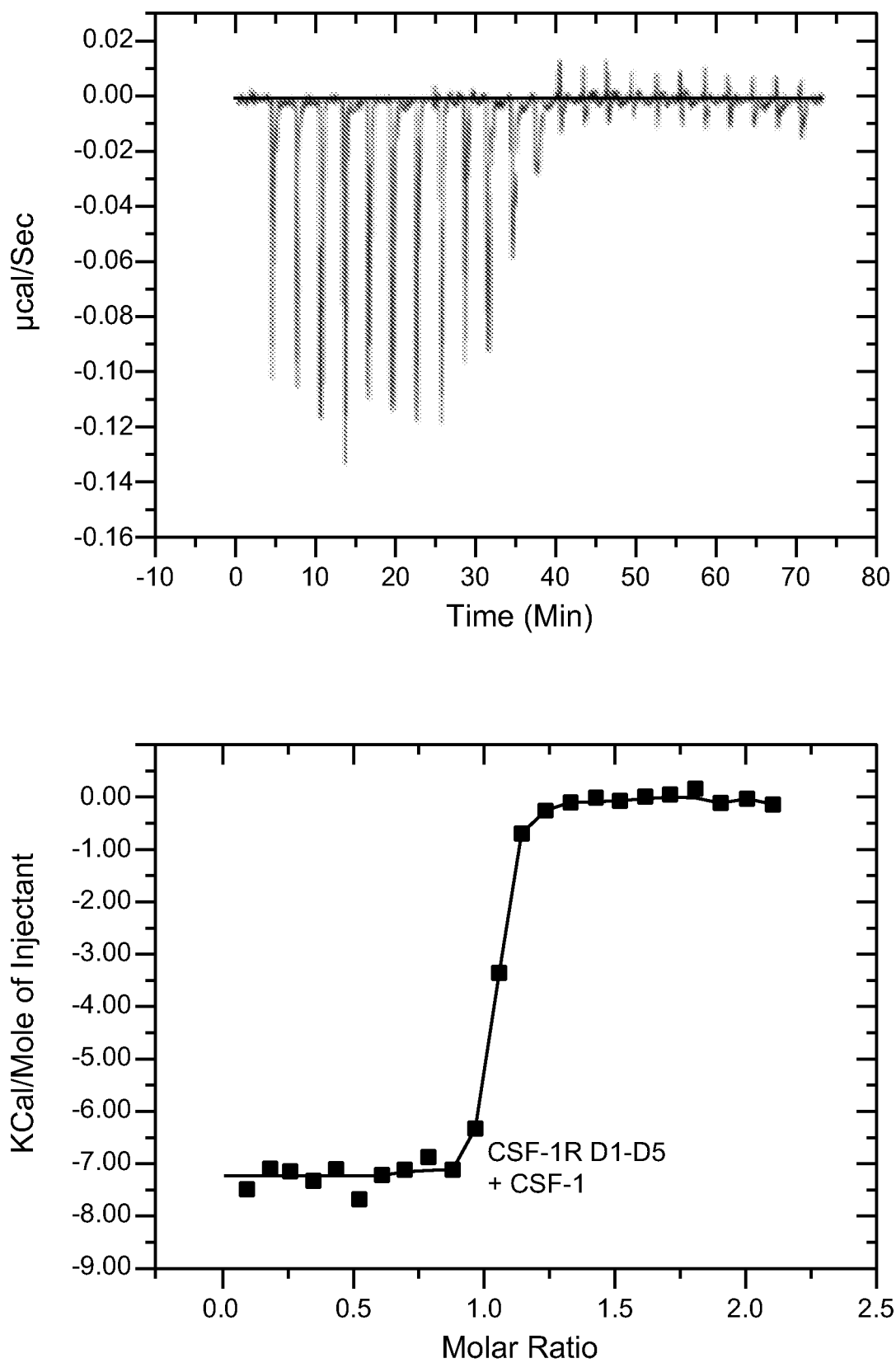
**FIG. 2B-2**

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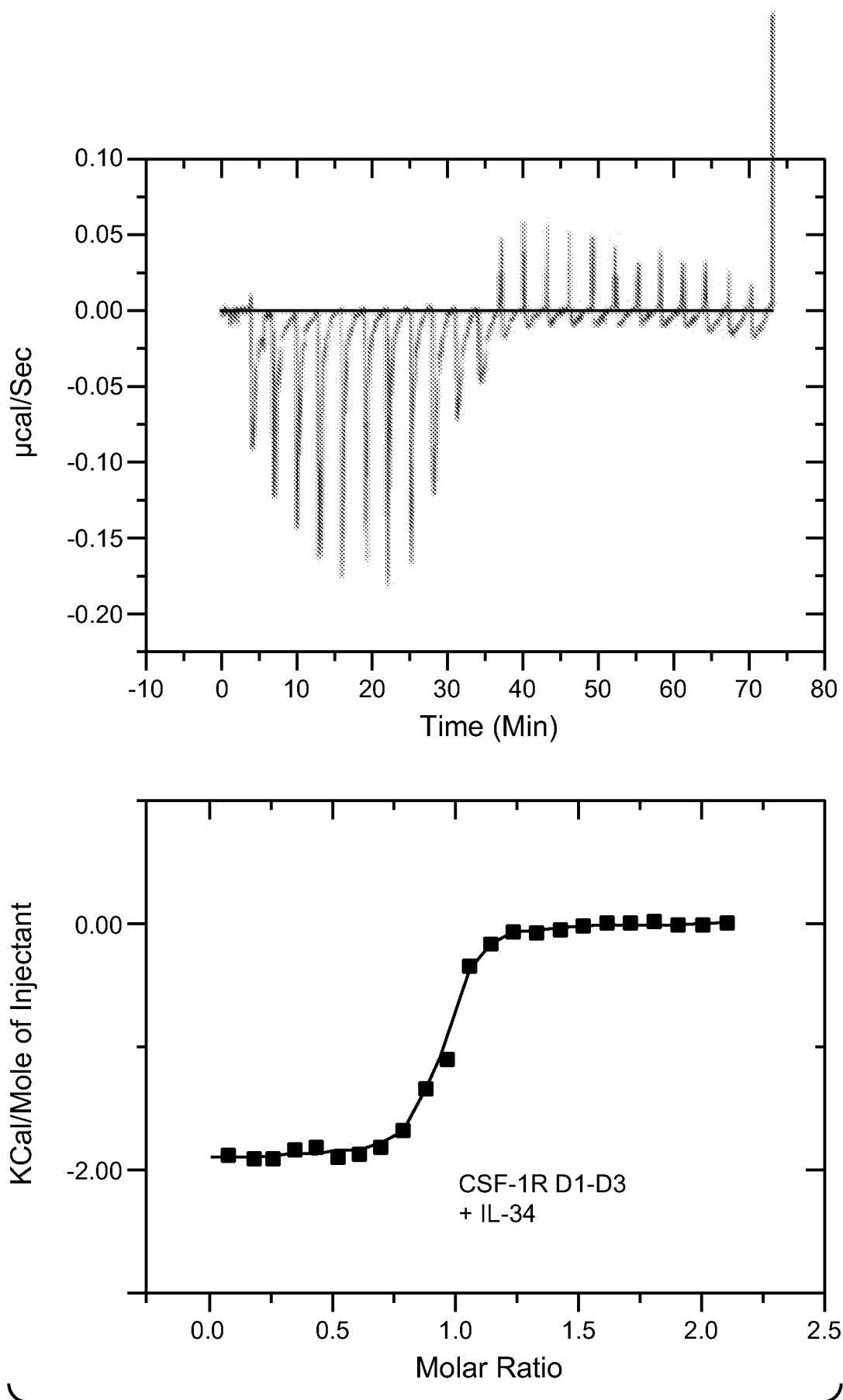
**FIG. 2B-3**



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**FIG. 2C-1**

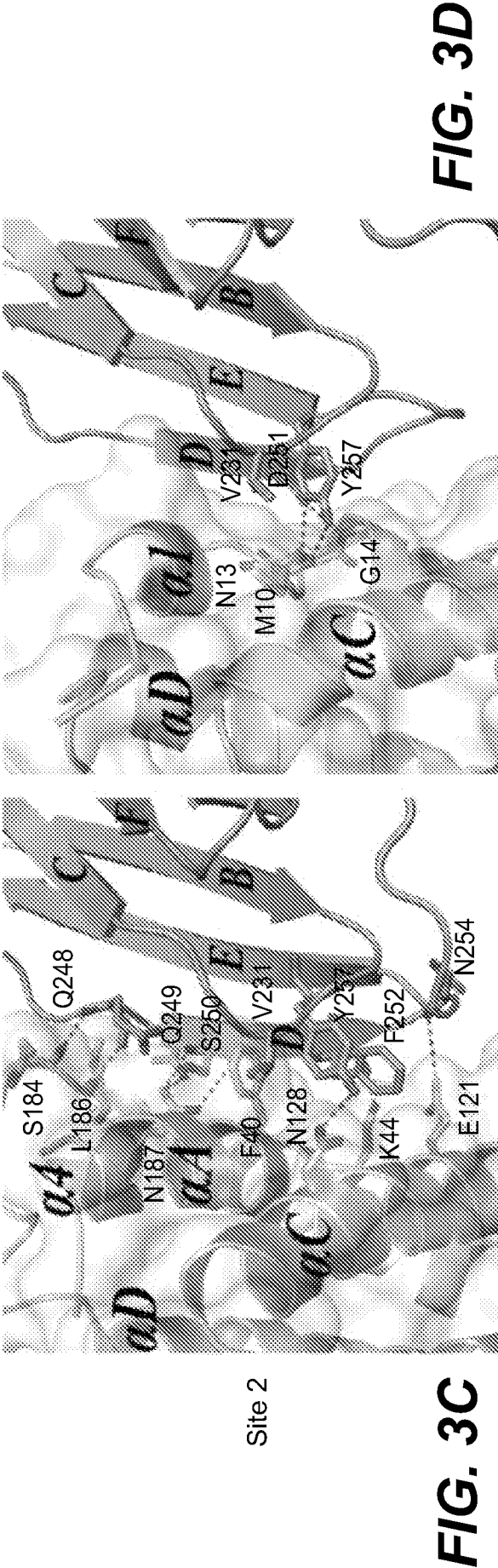
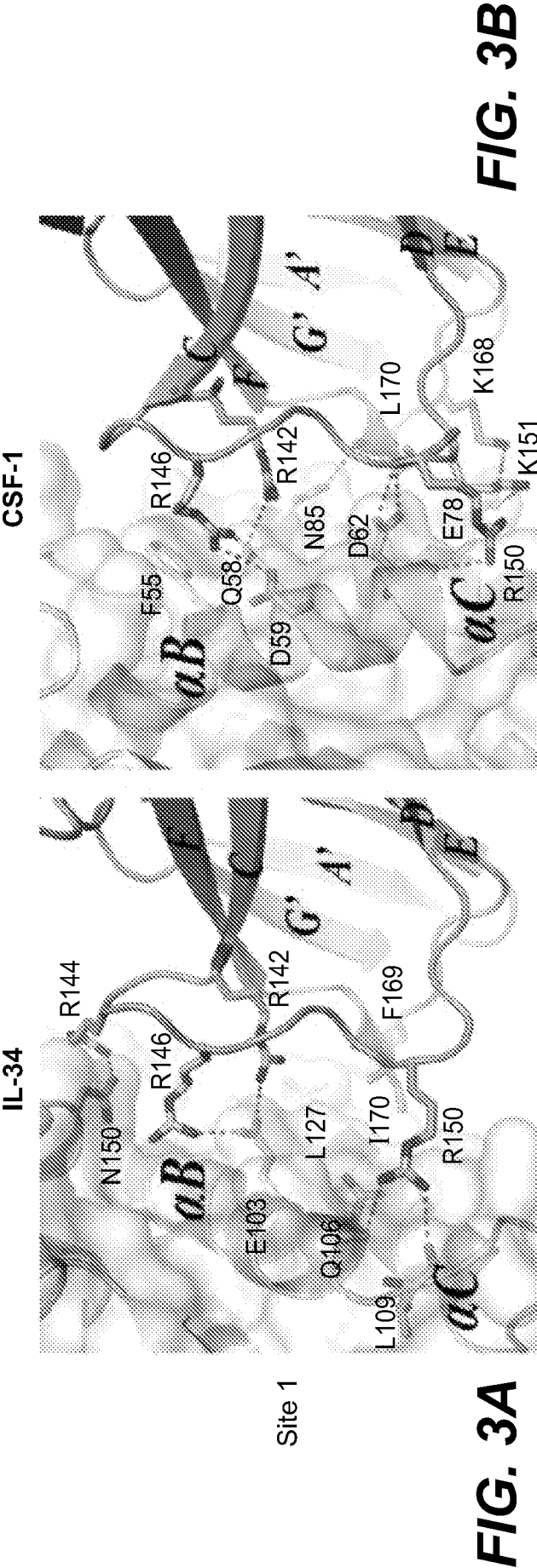
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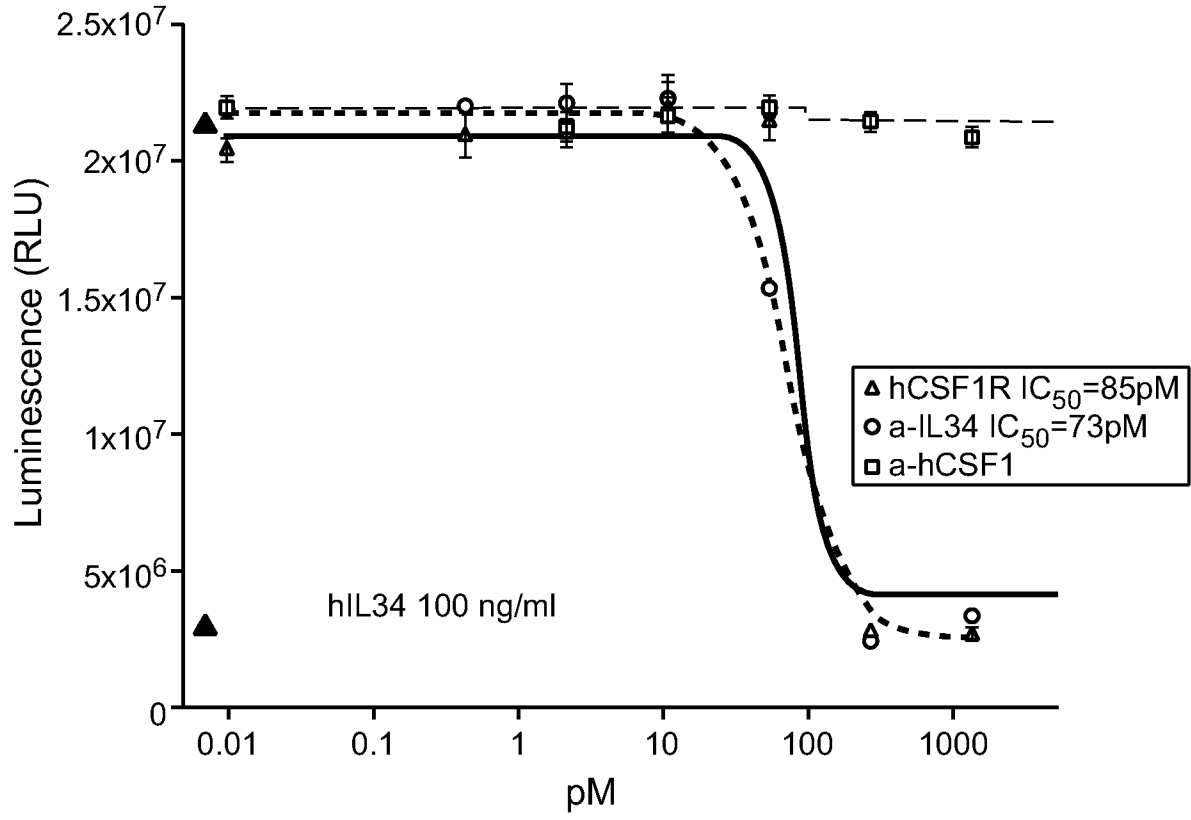
**FIG. 2C-2**

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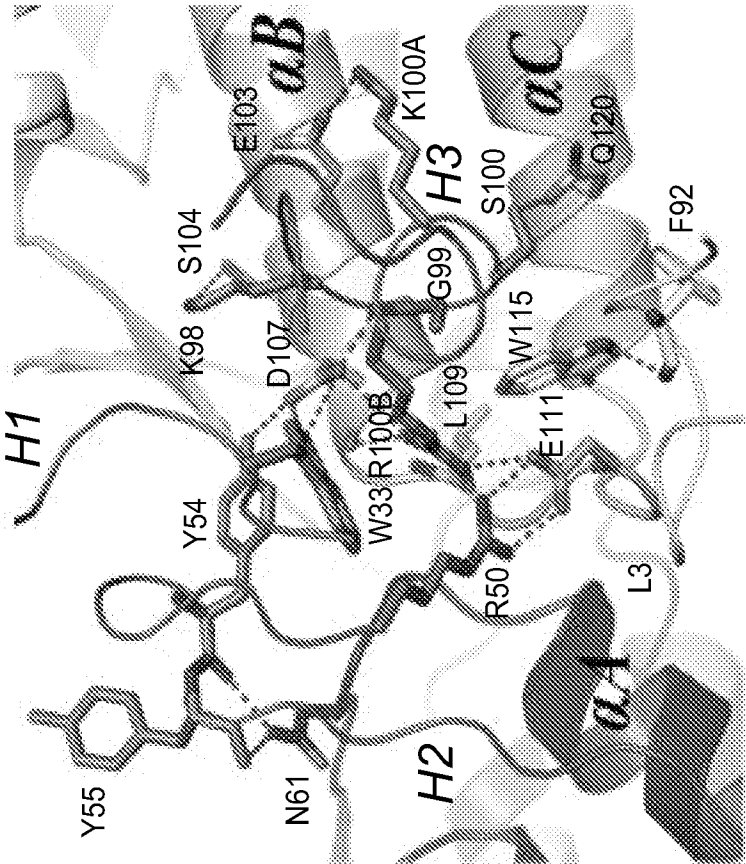
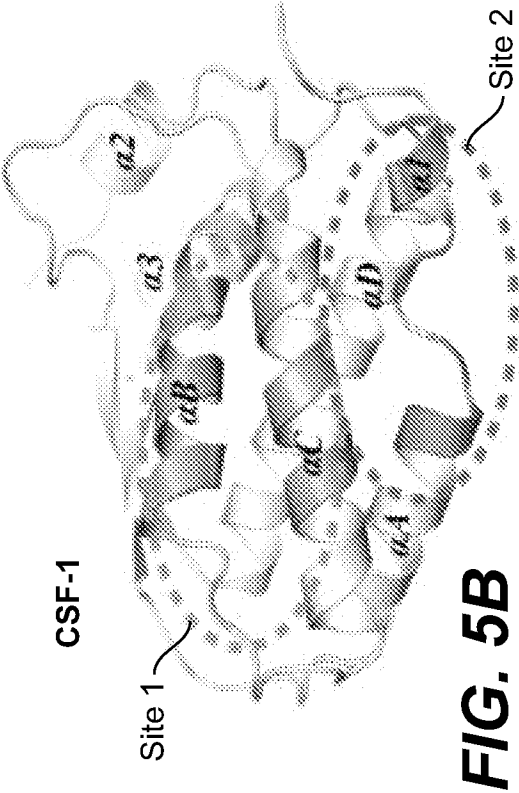
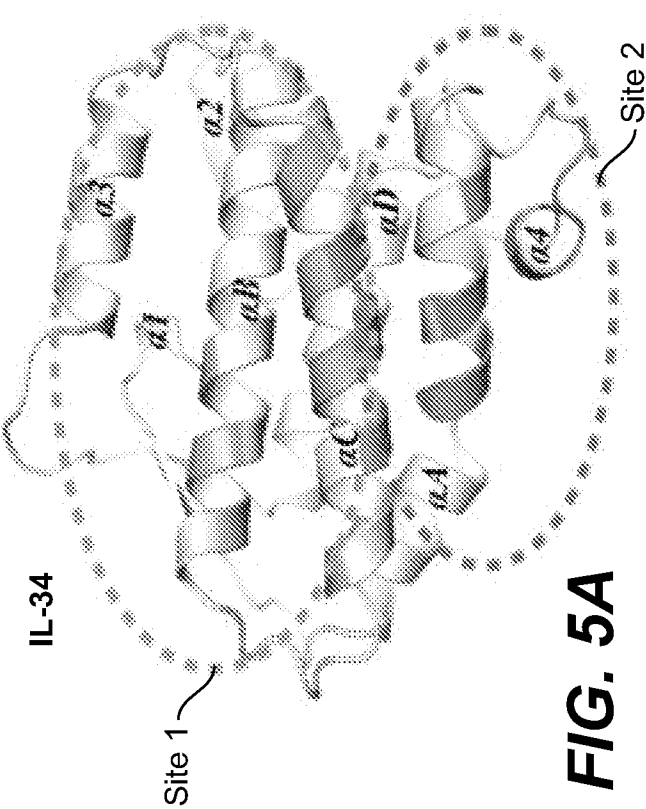
Injectant	Cell	$\Delta H$ (kcal/mol)	$\Delta S$ (cal/mol/K)	$\Delta G$ (Kcal/mol)	$K_d$ (nM)	N
CSF-1R D1-D5 (200 $\mu$ M)	hIL-34s (20 $\mu$ M)	-15.1	N.D.	N.D.	N.D.	0.89
CSF-1R D1-D3 (400 $\mu$ M)	hIL-34s (40 $\mu$ M)	-6.8	9.5	-9.6	94.3	0.94
CSF-1R D1-D5 (150 $\mu$ M)	hIL-34s (15 $\mu$ M) + CSF-1R D1-D3 (20 $\mu$ M)	-15.7	-12.5	-12.0	1.6	0.94
CSF-1R D1-D5 (200 $\mu$ M)	hCSF-1 (20 $\mu$ M)	-7.3	10.7	-10.5	20.2	1.00
CSF-1R D1-D3 (2000 $\mu$ M)	hCSF-1 (200 $\mu$ M)	-1.9	21.2	-8.2	892.9	0.93

**FIG. 2D**





**FIG. 4A**



**FIG. 4B**

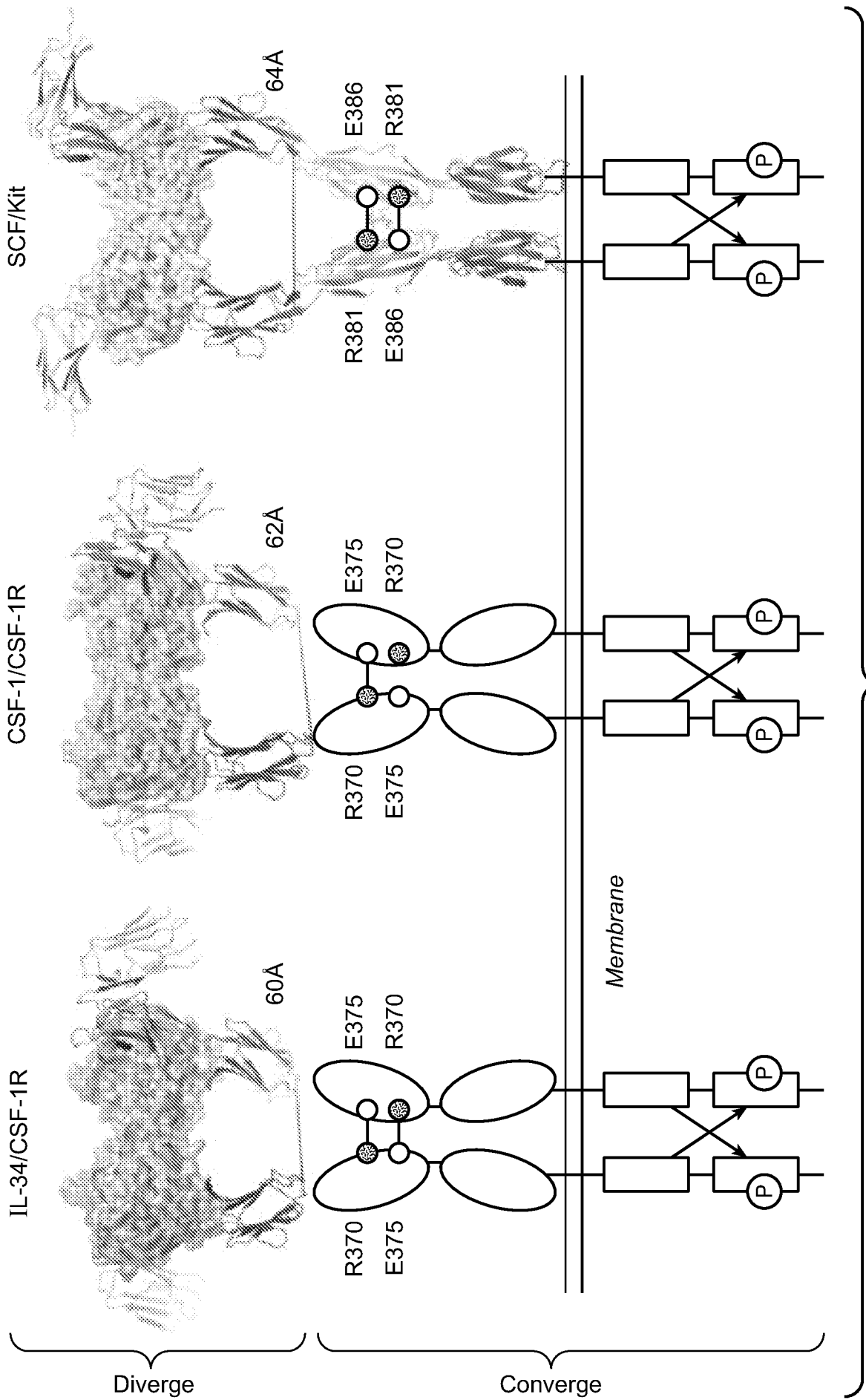


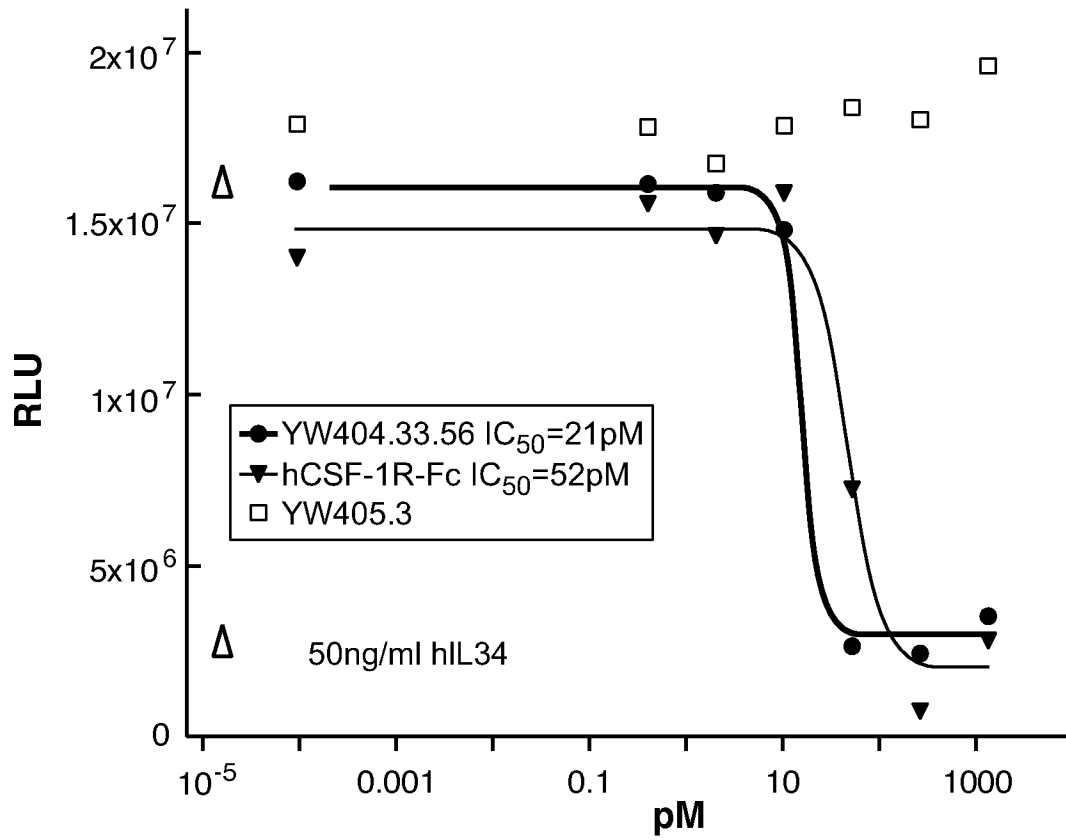
FIG. 6

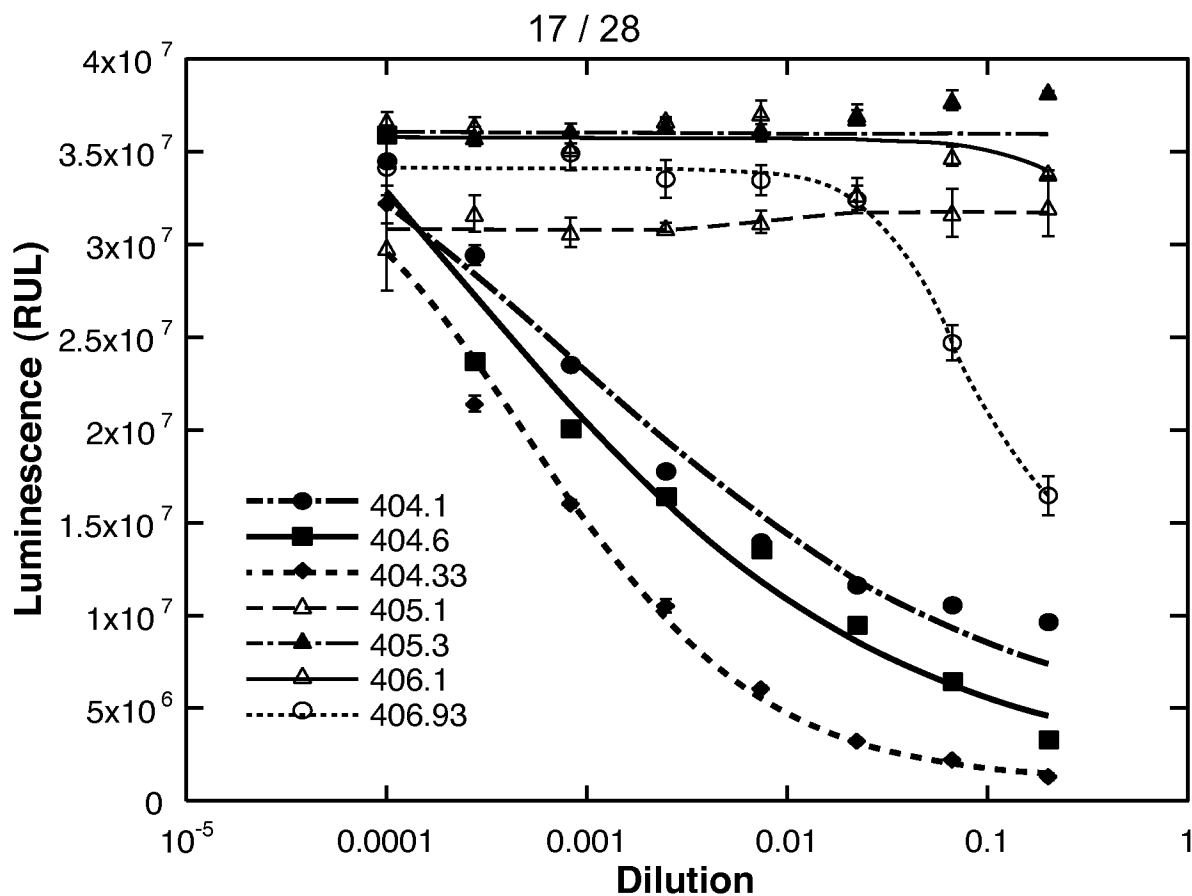
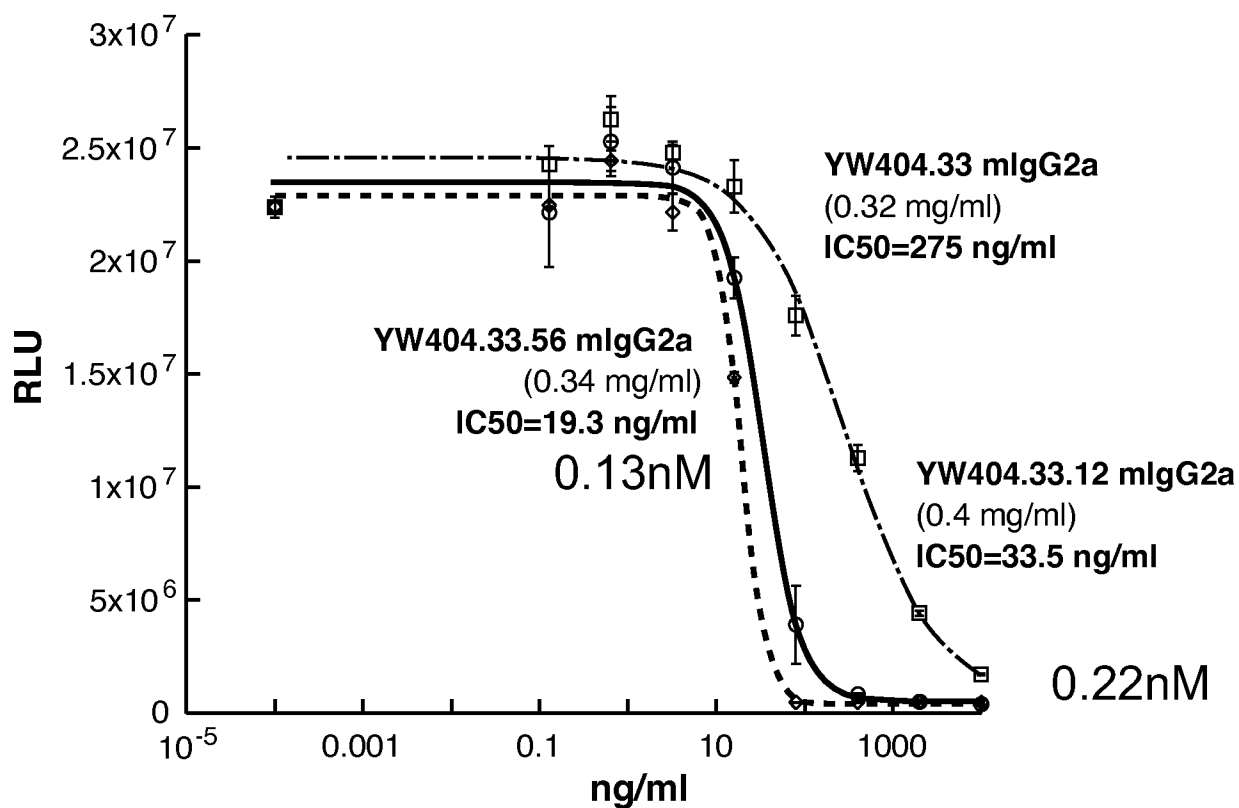






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**FIG. 8**

**FIG. 9A****FIG. 9B**

Kabat#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40			
	Chothia - CDR H1																																										
	Kabat - CDR H1																																										
404.1	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	S	L	R	L	S	C	A	A	S	G	F	T	F	T	S	N	Y	I	H	W	V	R	Q	A				
404.6	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	S	L	R	L	S	C	A	A	S	G	F	T	F	T	S	N	W	I	H	W	V	R	Q	A				
405.3	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	S	L	R	L	S	C	A	A	S	G	F	T	F	T	S	Y	G	I	H	W	V	R	Q	A				
404.33	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	S	L	R	L	S	C	A	A	S	G	F	T	F	T	S	T	W	I	H	W	V	R	Q	A				
404.33.10	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	S	L	R	L	S	C	A	A	S	G	F	T	F	T	S	T	W	I	H	W	V	R	Q	A				
404.33.12	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	S	L	R	L	S	C	A	A	S	G	F	T	F	T	S	T	W	I	H	W	V	R	Q	A				
404.33.11	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	S	L	R	L	S	C	A	A	S	G	F	T	F	T	S	T	W	I	H	W	V	R	Q	A				
404.33.56	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	S	L	R	L	S	C	A	A	S	G	F	T	F	T	S	T	W	I	H	W	V	R	Q	A				
404.33.93	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	S	L	R	L	S	C	A	A	S	G	F	T	F	T	S	T	W	I	H	W	V	R	Q	A				
Kabat#	41	42	43	44	45	46	47	48	49	50	51	52	A	B	C	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78		
	Chothia - CDR H2																																										
	Kabat - CDR H2																																										
	Chothia - CDR H2																																										
404.1	P	G	K	G	L	E	W	V	V	A	S	I	T	P	A	N	S	G	G	D	T	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	T	S	K	N	T	A
404.6	P	G	K	G	L	E	W	V	V	A	S	I	T	P	A	N	S	G	G	D	T	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	T	S	K	N	T	A
405.3	P	G	K	G	L	E	W	V	V	A	S	I	T	P	A	N	S	G	G	D	T	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	T	S	K	N	T	A
404.33	P	G	K	G	L	E	W	V	V	A	S	I	T	P	A	N	S	G	G	D	T	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	T	S	K	N	T	A
404.33.10	P	G	K	G	L	E	W	V	V	A	S	I	T	P	A	N	S	G	G	D	T	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	T	S	K	N	T	A
404.33.12	P	G	K	G	L	E	W	V	V	A	S	I	T	P	A	N	S	G	G	D	T	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	T	S	K	N	T	A
404.33.11	P	G	K	G	L	E	W	V	V	A	S	I	T	P	A	N	S	G	G	D	T	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	T	S	K	N	T	A
404.33.56	P	G	K	G	L	E	W	V	V	A	S	I	T	P	A	N	S	G	G	D	T	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	T	S	K	N	T	A
404.33.93	P	G	K	G	L	E	W	V	V	A	S	I	T	P	A	N	S	G	G	D	T	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	T	S	K	N	T	A
Kabat#	79	80	81	82	A	B	C	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	A	B	C	101	102	103	104	105	106	107	108	109	110	111	112	113		
	Chothia - CDR H3																																										
	Kabat - CDR H3																																										
	Chothia - CDR H3																																										
404.1	Y	L	Q	M	N	S	L	R	R	A	E	D	T	T	A	V	Y	Y	C	A	R	R	S	G	R	M	S	R	G	A	F	Y	W	G	Q	G	T	L	V	T	V	S	S
404.6	Y	L	Q	M	N	S	L	R	R	A	E	D	T	T	A	V	Y	Y	C	A	R	R	S	G	R	M	S	R	G	A	F	Y	W	G	Q	G	T	L	V	T	V	S	S
405.3	Y	L	Q	M	N	S	L	R	R	A	E	D	T	T	A	V	Y	Y	C	A	R	R	S	G	R	M	S	R	G	A	F	Y	W	G	Q	G	T	L	V	T	V	S	S
404.33	Y	L	Q	M	N	S	L	R	R	A	E	D	T	T	A	V	Y	Y	C	A	R	R	S	G	R	M	S	R	G	A	F	Y	W	G	Q	G	T	L	V	T	V	S	S
404.33.10	Y	L	Q	M	N	S	L	R	R	A	E	D	T	T	A	V	Y	Y	C	A	R	R	S	G	R	M	S	R	G	A	F	Y	W	G	Q	G	T	L	V	T	V	S	S
404.33.12	Y	L	Q	M	N	S	L	R	R	A	E	D	T	T	A	V	Y	Y	C	A	R	R	S	G	R	M	S	R	G	A	F	Y	W	G	Q	G	T	L	V	T	V	S	S
404.33.11	Y	L	Q	M	N	S	L	R	R	A	E	D	T	T	A	V	Y	Y	C	A	R	R	S	G	R	M	S	R	G	A	F	Y	W	G	Q	G	T	L	V	T	V	S	S
404.33.56	Y	L	Q	M	N	S	L	R	R	A	E	D	T	T	A	V	Y	Y	C	A	R	R	S	G	R	M	S	R	G	A	F	Y	W	G	Q	G	T	L	V	T	V	S	S
404.33.93	Y	L	Q	M	N	S	L	R	R	A	E	D	T	T	A	V	Y	Y	C	A	R	R	S	G	R	M	S	R	G	A	F	Y	W	G	Q	G	T	L	V	T	V	S	S

FIG. 10A

Kabat#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	A	B	C	D	E	F	28	29	30	31	32	33	34	35	36	37		
	Kabat - CDR L1																																												
	Chothia - CDR L1																																												
	Contact - CDR L1																																												
404.1	D	I	I	Q	M	T	Q	S	P	S	S	S	L	S	A	S	V	G	D	R	V	T	I	C	R	A	S	Q				D	V	S	T	A	V	A	W	Y	Q				
404.6	D	I	Q	Q	M	T	Q	S	P	S	S	S	L	S	A	S	V	G	D	R	V	T	I	C	R	A	S	Q				D	V	S	T	A	V	A	W	Y	Q				
405.3	D	I	Q	Q	M	T	Q	S	P	S	S	S	L	S	A	S	V	G	D	R	V	T	I	C	R	A	S	Q				D	V	S	T	A	V	A	W	Y	Q				
404.33	D	I	Q	Q	M	T	Q	S	P	S	S	S	L	S	A	S	V	G	D	R	V	T	I	C	R	A	S	Q				D	V	S	T	A	V	A	W	Y	Q				
404.33.10	D	I	Q	Q	M	T	Q	S	P	S	S	S	L	S	A	S	V	G	D	R	V	T	I	C	R	A	S	Q				D	V	S	T	A	V	A	W	Y	Q				
404.33.12	D	I	Q	Q	M	T	Q	S	P	S	S	S	L	S	A	S	V	G	D	R	V	T	I	C	R	A	S	Q				D	V	S	T	A	V	A	W	Y	Q				
404.33.11	D	I	Q	Q	M	T	Q	S	P	S	S	S	L	S	A	S	V	G	D	R	V	T	I	C	R	A	S	Q				D	V	S	T	A	V	A	W	Y	Q				
404.33.56	D	I	Q	Q	M	T	Q	S	P	S	S	S	L	S	A	S	V	G	D	R	V	T	I	C	R	A	S	Q				D	V	S	T	A	V	A	W	Y	Q				
404.33.93	D	I	Q	Q	M	T	Q	S	P	S	S	S	L	S	A	S	V	G	D	R	V	T	I	C	R	A	S	Q				D	V	S	T	A	V	A	W	Y	Q				
Kabat#	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80		
	Kabat - CDR L2																																												
	Chothia - CDR L2																																												
	Contact - CDR L2																																												
404.1	Q	K	P	G	K	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	F	D	T	L	T	I	I	I	Q	Q	Q	P			
404.6	Q	K	P	G	K	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	F	D	T	L	T	I	I	I	Q	Q	Q	P			
405.3	Q	K	P	G	K	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	F	D	T	L	T	I	I	I	Q	Q	Q	P			
404.33	Q	K	P	G	K	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	F	D	T	L	T	I	I	I	Q	Q	Q	P			
404.33.10	Q	K	P	G	K	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	F	D	T	L	T	I	I	I	Q	Q	Q	P			
404.33.12	Q	K	P	G	K	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	F	D	T	L	T	I	I	I	Q	Q	Q	P			
404.33.11	Q	K	P	G	K	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	F	D	T	L	T	I	I	I	Q	Q	Q	P			
404.33.56	Q	K	P	G	K	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	F	D	T	L	T	I	I	I	Q	Q	Q	P			
404.33.93	Q	K	P	G	K	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	F	D	T	L	T	I	I	I	Q	Q	Q	P			
Kabat#	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108																	
	Kabat - CDR L3																																												
	Chothia - CDR L3																																												
	Contact - CDR L3																																												
404.1	E	D	F	F	A	T	T	C	Q	Q	Q	S	Y	T	T	P	T	F	G	Q	T	T	K	V	I	K	R																		
404.6	E	D	F	F	A	T	T	C	Q	Q	Q	S	Y	T	T	P	T	F	G	Q	T	T	K	V	I	K	R																		
405.3	E	D	F	F	A	T	T	C	Q	Q	Q	S	Y	T	T	P	T	F	G	Q	T	T	K	V	I	K	R																		
404.33	E	D	F	F	A	T	T	C	Q	Q	Q	S	Y	T	T	P	T	F	G	Q	T	T	K	V	I	K	R																		
404.33.10	E	D	F	F	A	T	T	C	Q	Q	Q	S	Y	T	T	P	T	F	G	Q	T	T	K	V	I	K	R																		
404.33.12	E	D	F	F	A	T	T	C	Q	Q	Q	S	Y	T	T	P	T	F	G	Q	T	T	K	V	I	K	R																		
404.33.11	E	D	F	F	A	T	T	C	Q	Q	Q	S	Y	T	T	P	T	F	G	Q	T	T	K	V	I	K	R																		
404.33.56	E	D	F	F	A	T	T	C	Q	Q	Q	S	Y	T	T	P	T	F	G	Q	T	T	K	V	I	K	R																		
404.33.93	E	D	F	F	A	T	T	C	Q	Q	Q	S	Y	T	T	P	T	F	G	Q	T	T	K	V	I	K	R																		

FIG. 10B

FIG. 10B

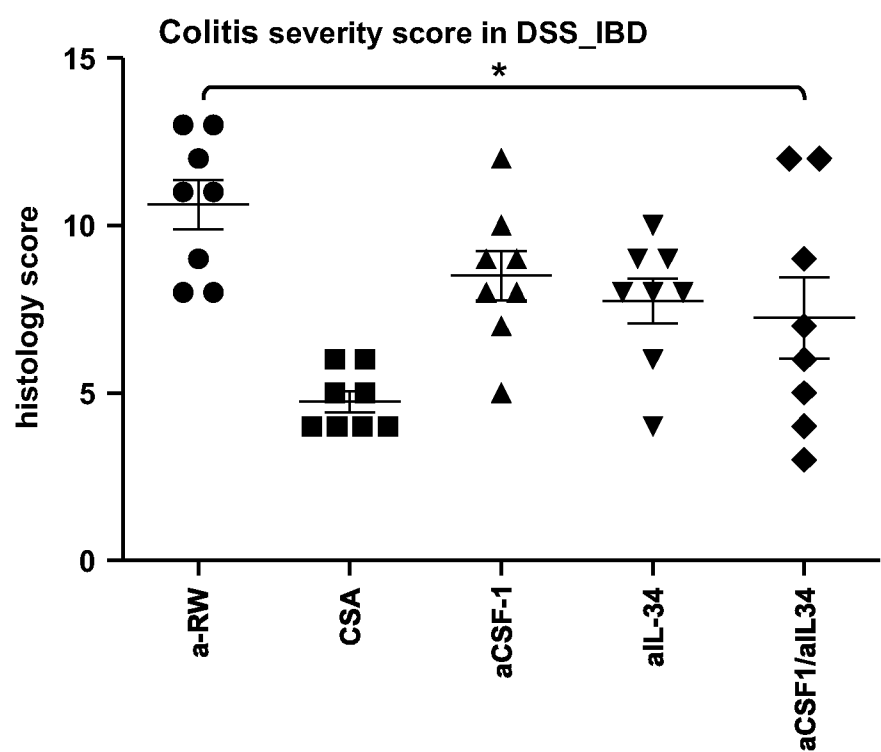
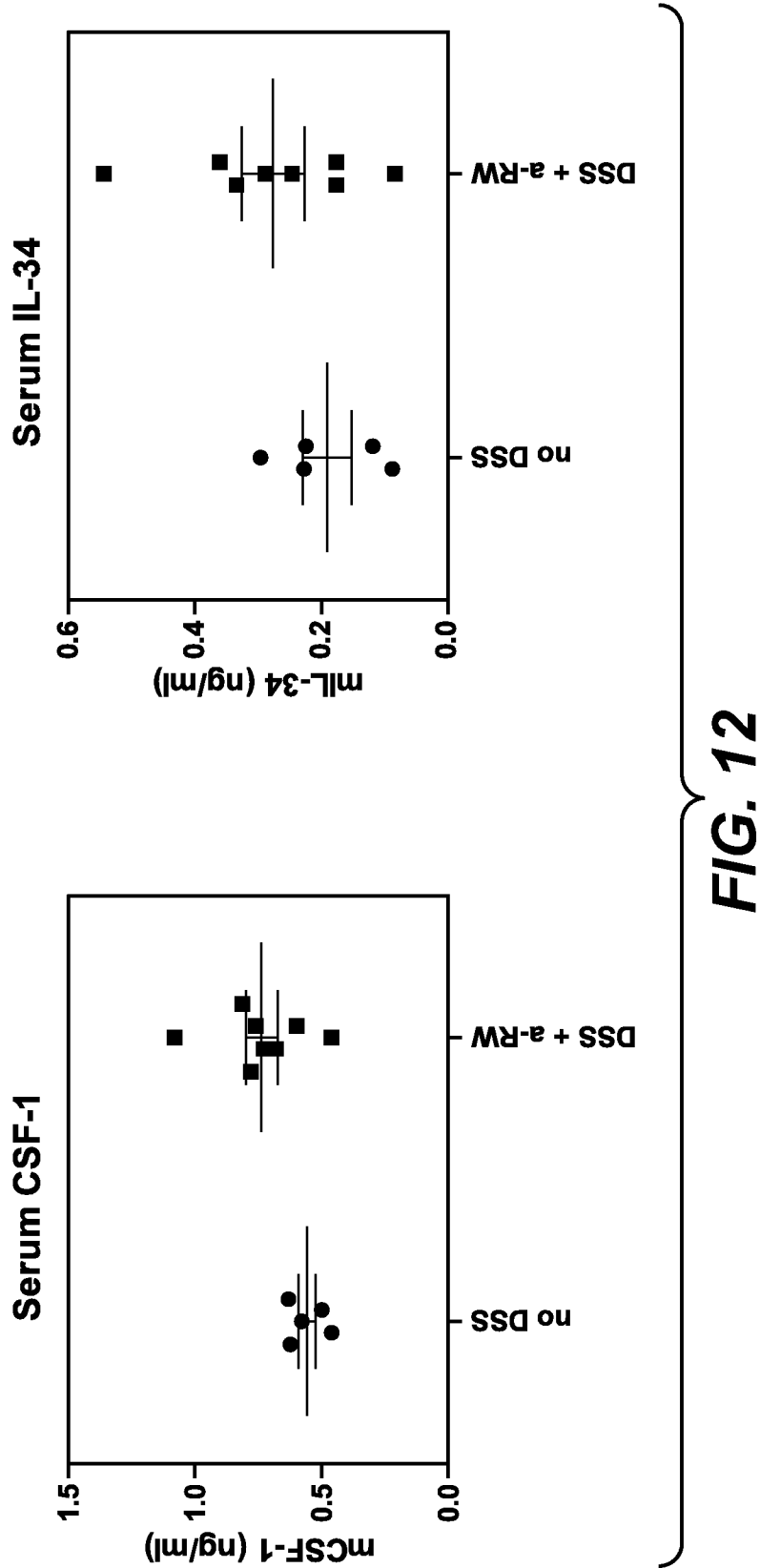


FIG. 11



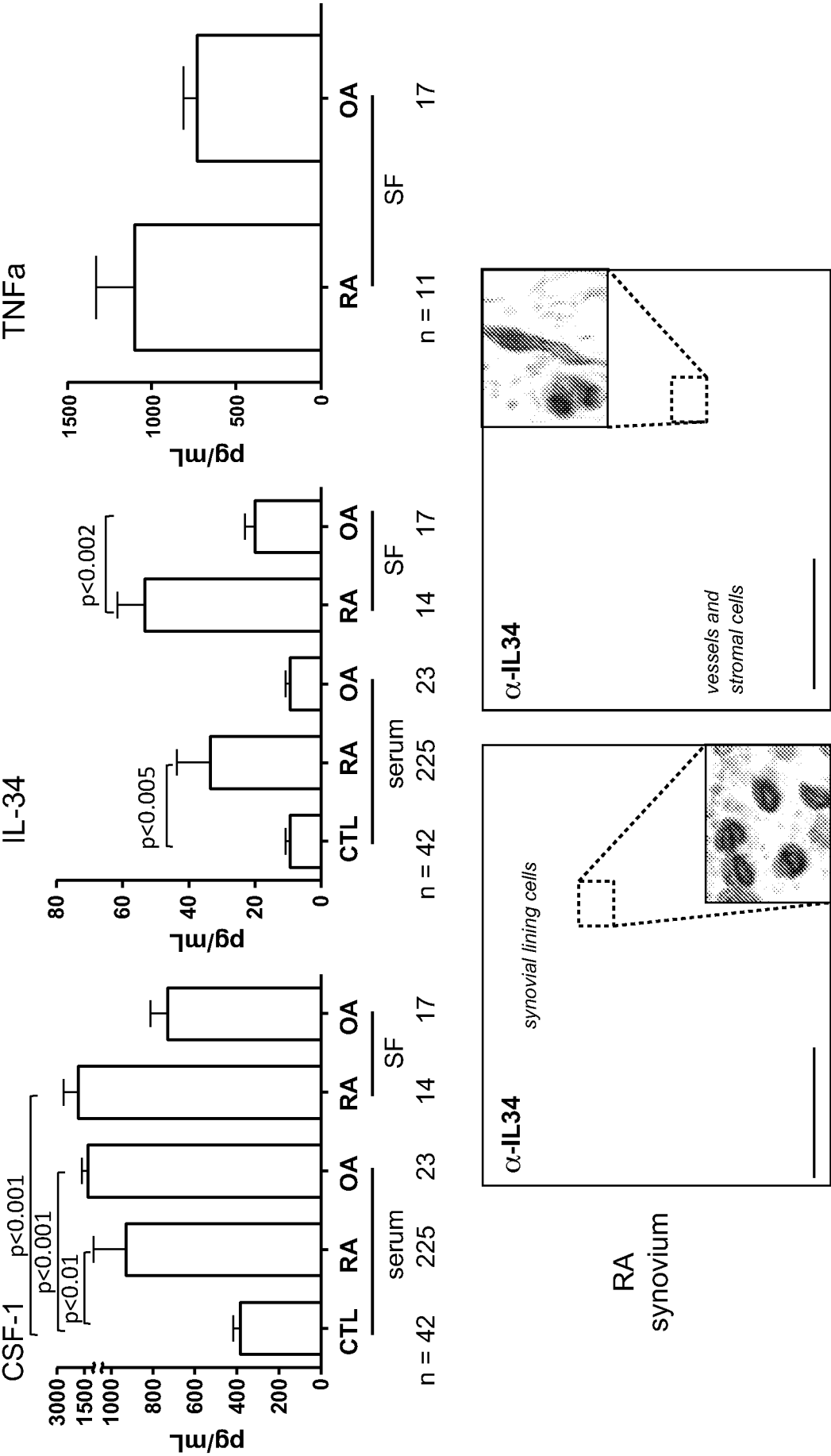


FIG. 13



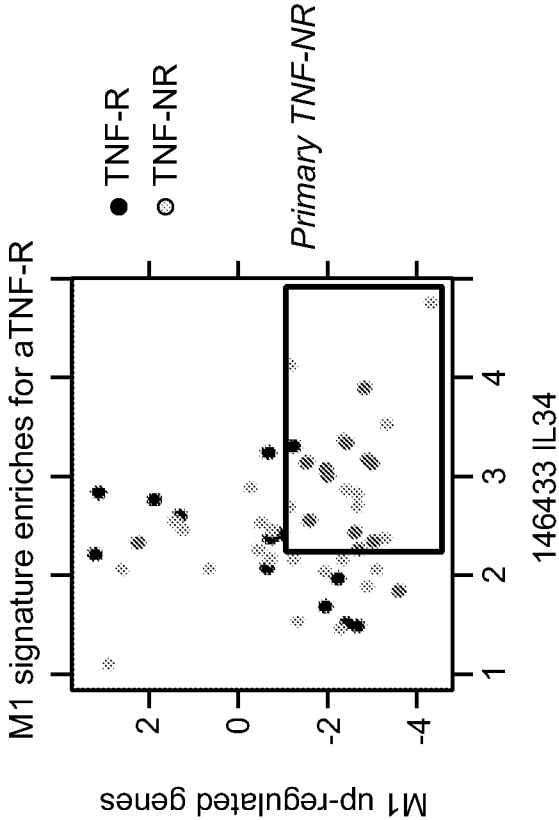


FIG. 14A

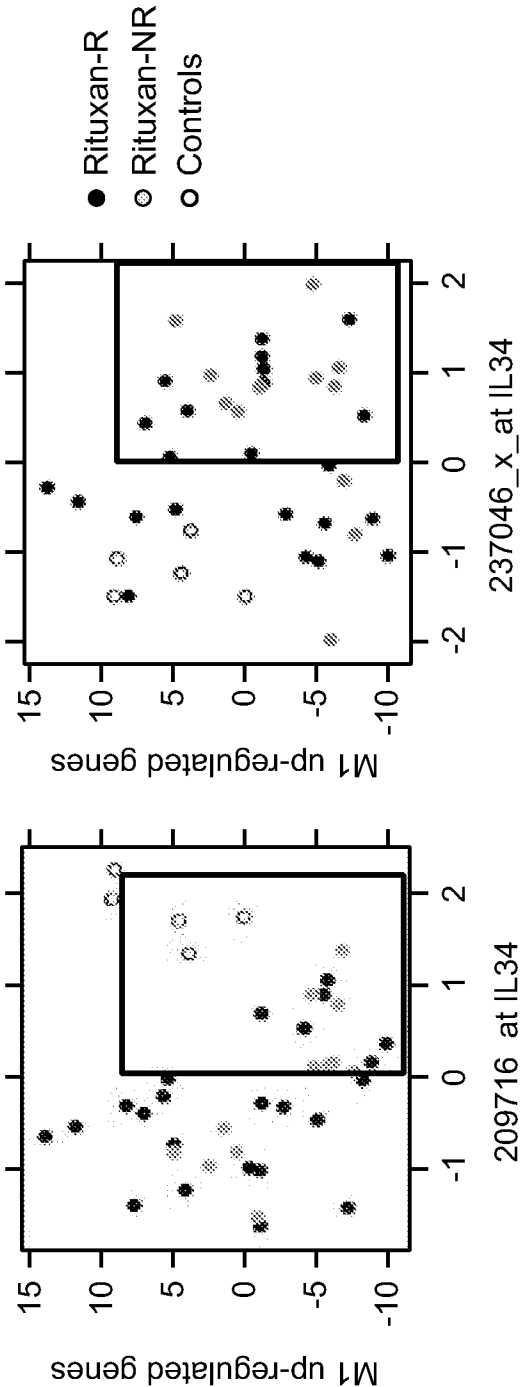


FIG. 14B

ALL secondary TNF-NR

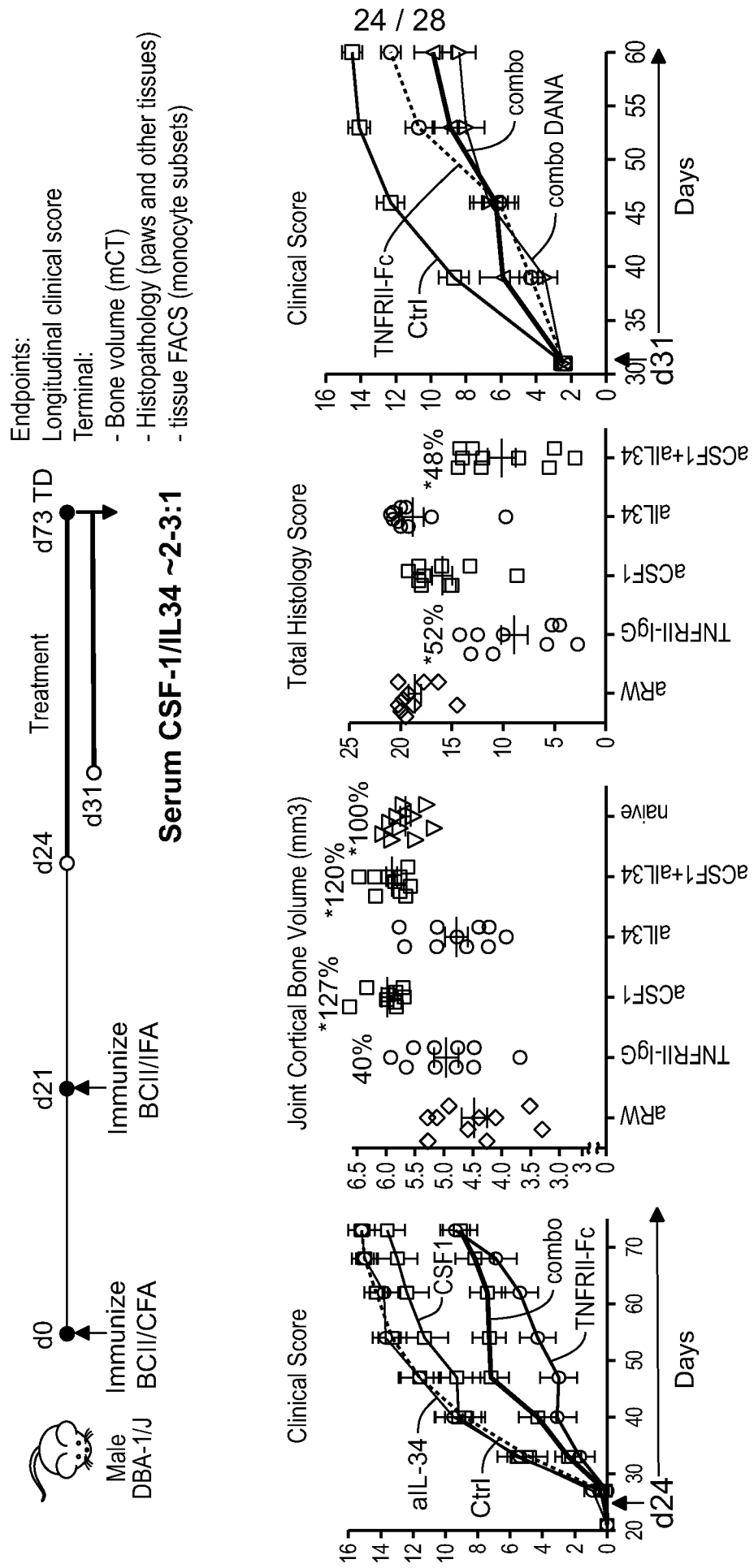
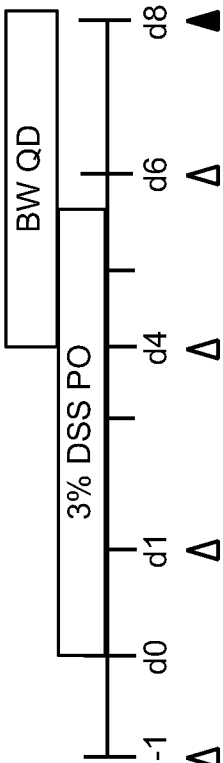


FIG. 15

C57BL/6J  
Female  
6-8 wk old



Treatment d-1



Colon histology (total)

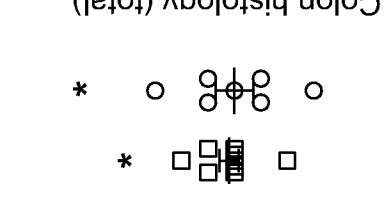
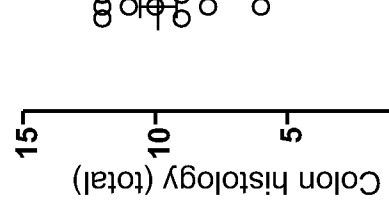
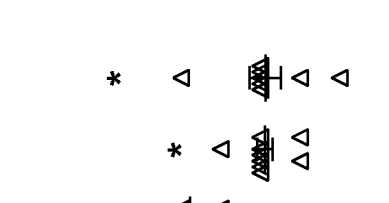
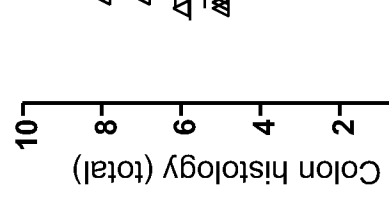
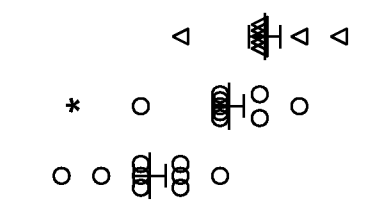
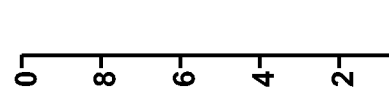


FIG. 16A

Colon histology (total)

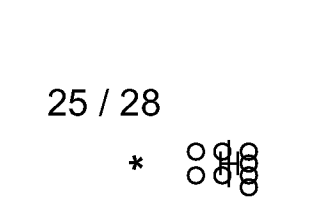
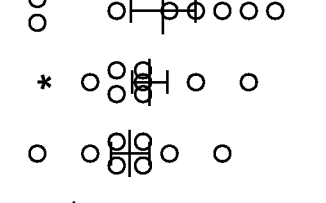
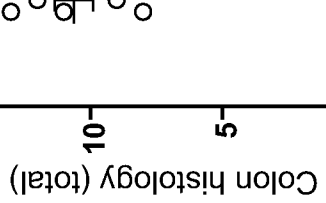
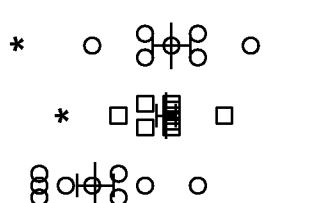
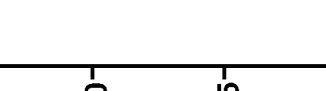


FIG. 16B

Colon histology (total)

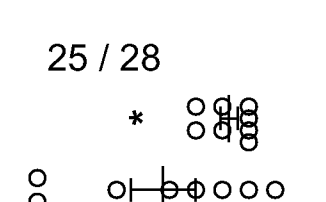
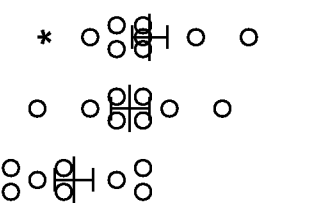
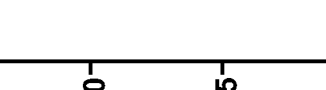
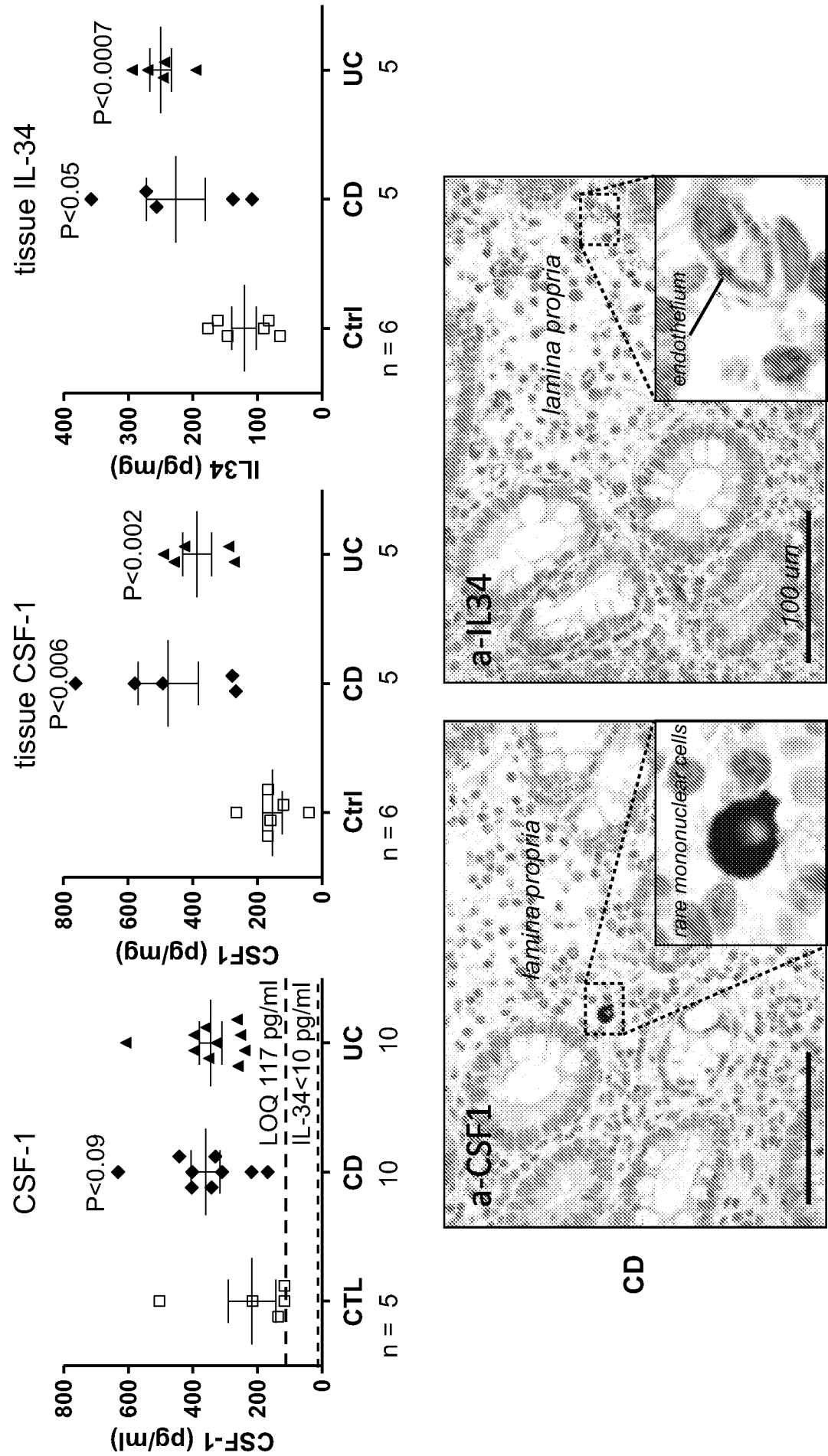


FIG. 16C

Serum CSF-1/IL34 ~3-4:1

25 / 28



**FIG. 17**

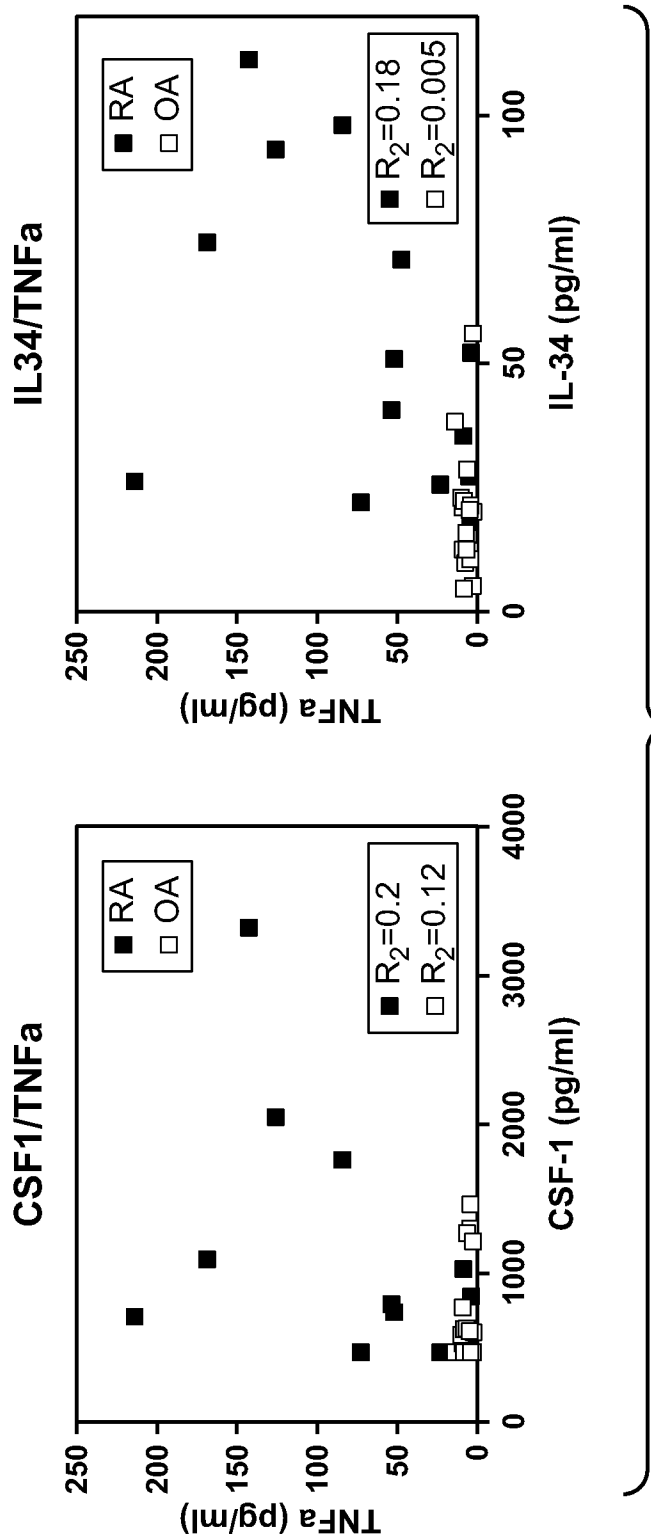
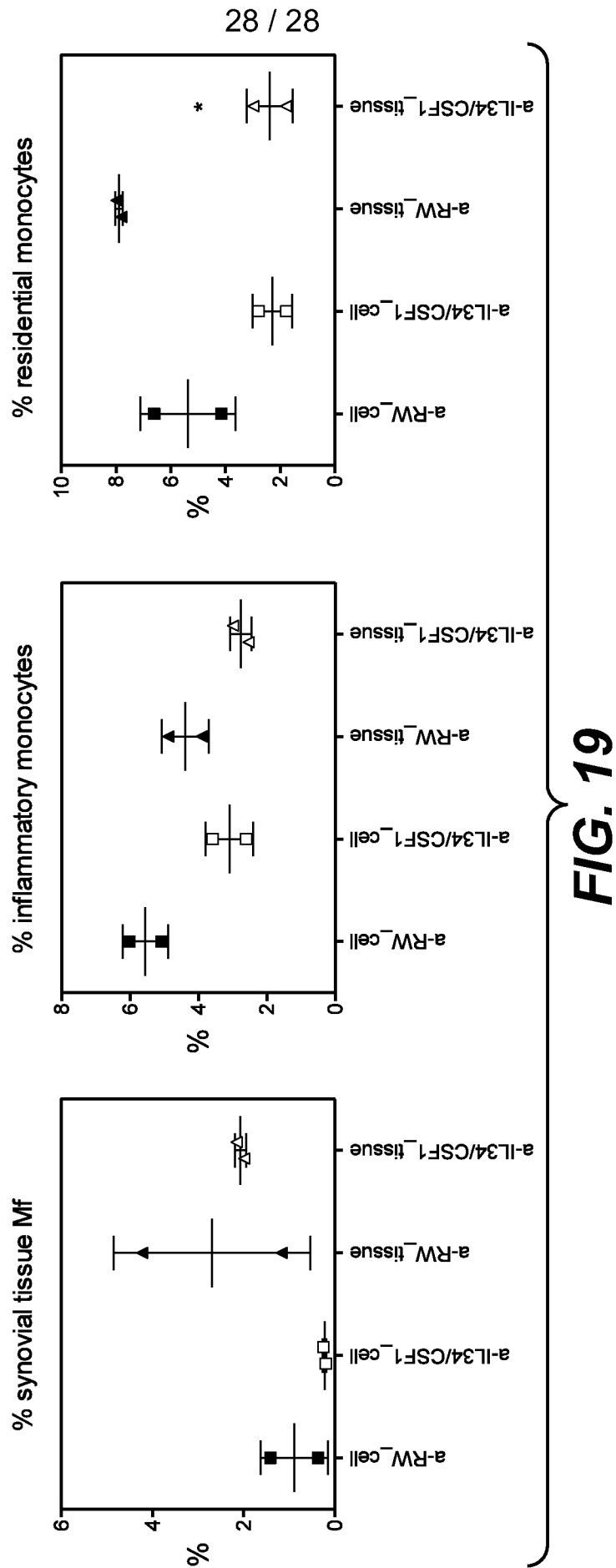


FIG. 18



## INTERNATIONAL SEARCH REPORT

 International application No.  
**PCT/US2013/024998**

## A. CLASSIFICATION OF SUBJECT MATTER

**C07K 16/24 (2006.01) A61K 39/395 (2006.01) A61P 19/02 (2006.01)**

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed		"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
Date of the actual completion of the international search 17 May 2013		Date of mailing of the international search report 17 May 2013	
<b>Name and mailing address of the ISA/AU</b>  AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au Facsimile No.: +61 2 6283 7999		<b>Authorised officer</b>  Anita Premkumar AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0399359636	

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

**See Supplemental Box for Details**

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
**1-56, 81-84 and 89 (completely) and claims 68-80, 85-88 and 90-92 (partially)**

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.



INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/US2013/024998
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	MA, X et al, STRUCTURE (April 2012), Vol 20, pages 676-687. "Structural Basis for the Dual Recognition of Helical Cytokines IL-34 and CSF-1 by CSF-1R." Abstract, 679 col 1 para 1, page 681, 686 col 1 para 1, Table S3, Supplemental Experimental Procedures	1-12, 45-49, 52-56 and 68-90
A	US 2011/0243947 A1 (DOODY et al) 06 October 2011	
A	CHIHARA. T et al, Cell Death and Differentiation (2010), Vol 17, pages 1917-1927. "IL-34 and M-CSF share the receptor Fms but are not identical in biological activity and signal activation."	
A	HWANG. S-J et al, Arthritis Research & Therapy (2012), Vol 14, R14, pages 1-10 "Interleukin-34 produced by human fibroblast-like synovial cells in rheumatoid arthritis supports osteoclastogenesis."	

**Supplemental Box****Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-56, 81-84 and 89 (completely) and claims 68-80, 85-88 and 90-92 (partially) are directed to antibodies that bind IL-34. The feature of an antibody binding IL-34 is specific to this group of claims.
- Claims 57-67 (completely) and claims 68-80, 85-88 and 90-92 (partially) are directed to antibodies that bind CSF-1R. The feature of an antibody binding CSF-1R is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

The claims are directed to two antibodies to two separate proteins - IL-34 and CSF-1R.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

<b>INTERNATIONAL SEARCH REPORT</b> Information on patent family members		International application No. <b>PCT/US2013/024998</b>	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
US 2011/0243947 A1	06 Oct 2011	AR 080698 A1	02 May 2012
		AU 2011232850 A1	06 Sep 2012
		CA 2795081 A1	06 Oct 2011
		CN 102834414 A	19 Dec 2012
		EA 201270732 A1	30 Jan 2013
		EP 2552966 A1	06 Feb 2013
		KR 20130001258 A	03 Jan 2013
		MX 2012011234 A	30 Nov 2012
		SG 183829 A1	30 Oct 2012
		TW 201138824 A	16 Nov 2011
		US 2011243947 A1	06 Oct 2011
		US 8263079 B2	11 Sep 2012
		WO 2011123381 A1	06 Oct 2011
<b>End of Annex</b>			
<p>Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.</p> <p>Form PCT/ISA/210 (Family Annex)(July 2009)</p>			