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(54) Title: CSF1R BINDING AGENTS

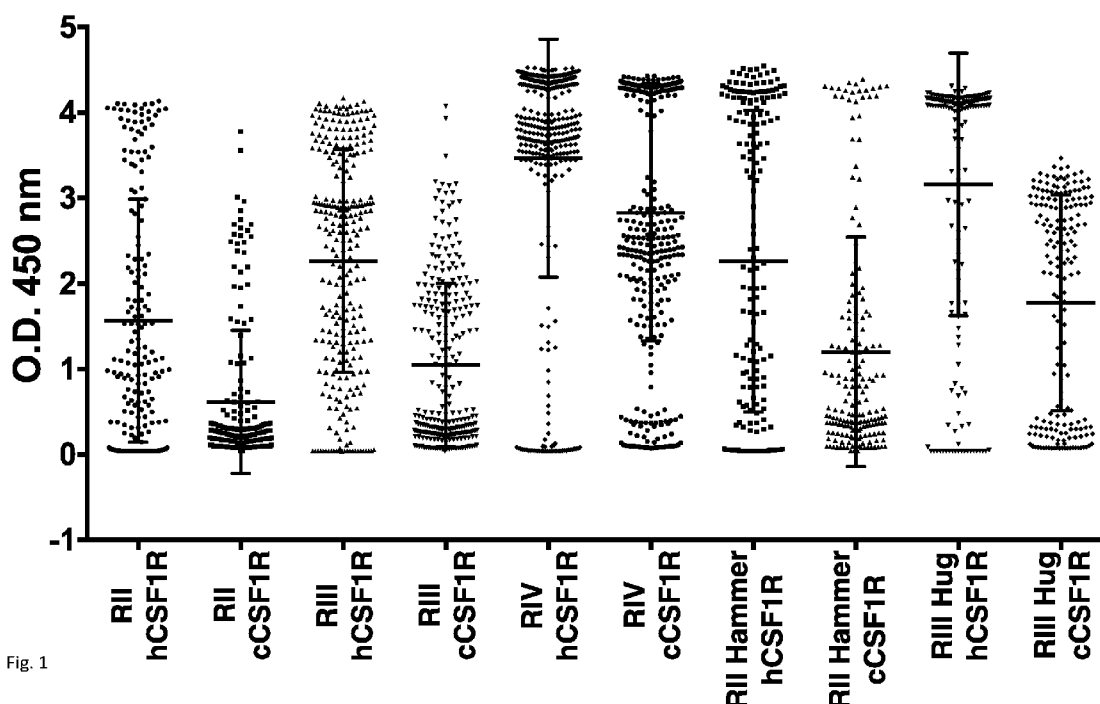


Fig. 1

(57) Abstract: Provided herein are antibody molecules that bind specifically to Colony Stimulating Factor 1 Receptor (CSF1R) and related nucleic acid molecules, vectors and host cells. Also provided herein are medical uses of such antibody molecules.

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CSF1R BINDING AGENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of GB Patent Application No. 1819045.4, filed on November 22, 2018, GB Patent Application No. 1810226.9, filed on June 21, 2018, and GB Patent Application No. 1803226.8, filed on February 28, 2018, the disclosure of each of which is hereby incorporated by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: ULTL_001_01WO_SeqList_ST25.txt, date recorded: February 28, 2019, file size 158,222 bytes).

FIELD OF THE INVENTION

The invention relates to antibody molecules binding specifically to CSF1R (also known as Colony Stimulating Factor 1 Receptor, C-FMS, CD115, CSF-1R, CSFR, FIM2, FMS, HDLS, M-CSF-R) and medical uses thereof.

BACKGROUND OF THE INVENTION

CSF1R is a cell surface receptor that is a member of the immunoglobulin superfamily and is principally expressed on cells of mononuclear lineage, especially macrophages. CSF1R binds two known ligands, CSF1 (also known as M-CSF) and IL-34. The interaction of these ligands with CSF1R plays a direct role in the maturation, survival, proliferation and differentiation of mononuclear phagocytes, such as macrophages and monocytes.

While the role of CSF1R signalling in non-disease states is important for mononuclear cell regulation, it is also a key mechanism by which tumour cells recruit and maintain immunosuppressive tumour-associated macrophages (TAMs). Indeed, CSF1R has been shown to be highly expressed in TAMs in several cancers. In early stage and metastatic cancer the presence of intra-tumour CSF1R+ cells, including macrophages and neutrophils, correlates with poor survival. Hence, antagonistic anti-CSF1R mAbs have the potential to act as immunotherapeutic agents in cancer and immune disease settings, and to amplify the effectiveness of currently established therapies.

The majority of currently approved antibody therapeutics are derived from immunized rodents. Many of those antibodies have undergone a process known as “humanization”, via the “grafting” of murine CDRs into human v-gene framework sequences (see Nelson *et al.*, 2010, Nat Rev Drug Discov 9: 767-774). This process is often inaccurate and leads to a reduction in target binding affinity of the resulting antibody. To return the binding affinity of the original antibody, murine residues are usually introduced at key positions in the variable domain frameworks of the grafted v-domains (also known as “back-mutations”).

While antibodies humanized via CDR grafting and back mutations have been shown to induce lower immune response rates in the clinic in comparison to those with fully murine v-domains, antibodies humanized using this basic grafting method still carry significant clinical development risks due to the potential physical instability and immunogenicity motifs still housed in the grafted CDR loops. Antibodies such as CSF1R inhibitors that target receptors on antigen-presenting immune cells, and whose pharmacological function is to stimulate immune responses, are at heightened risk of provoking anti-drug antibody responses. These anti-drug antibody responses in the patient can reduce drug half-life, potency and safety during clinical use. As animal testing of protein immunogenicity is often non-predictive of immune responses in man, antibody engineering for therapeutic use focuses on minimizing predicted human T-cell epitope content, non-human germline amino acid content and aggregation potential in the purified protein.

The ideal humanized agonistic anti-CSF1R antibody would therefore have as many identical residues as possible in the v-domains to those found in both the frameworks and CDRs of well-characterized human germline sequences. Townsend *et al.* (2015; PNAS 112: 15354-15359) describe a method for generating antibodies in which CDRs derived from rat, rabbit and mouse antibodies were grafted into preferred human frameworks and then subjected to a human germ-lining approach termed “Augmented Binary Substitution”. Although the approach demonstrated a fundamental plasticity in the original antibody paratopes, even when an investigator is in possession of highly accurate antibody-antigen co-crystal structural data, it is still not possible to reliably predict which individual residues in the CDR loops of any given antibody can be converted to human germline, and in what combination. Additionally, the Townsend *et al.* study did not address the addition of mutagenesis beyond the residues found in the human germline at positions where the removal of development risk motifs might be beneficial. This is a technological limitation which renders the process inherently inefficient, requiring an extra stage of modification of the starting antibody sequence. In addition, it cannot currently be accurately predicted what modifications in distal

positions of the protein sequence of an individual v-domain, or even on the partner v-domain, might facilitate the removal of risk motifs while maintaining antigen binding affinity and specificity.

- 5 CDR germ-lining and development quality optimisation is thus a complex, multifactorial problem, as multiple functional properties of the molecule should preferably be maintained or improved, including in this instance: target binding specificity, CSF1R/CSF1 signalling antagonism, affinity to CSF1R from both human and animal test species (e.g. cynomolgus monkey, also known as the crab-eating macaque, i.e. *Macaca fascicularis*, and/or Rhesus
10 monkey, i.e. *Macaca mulatta*) should be as similar as possible to facilitate highly accurate preclinical safety testing, v-domain biophysical stability and/or IgG expression yield should be optimal for manufacturing purposes. Antibody engineering studies have shown that mutation of even single residue positions in key CDRs can have dramatic negative effects on all of these desired molecular properties.

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- WO2011/140249A2 describes an antagonistic murine anti-CSF1R IgG molecule termed "0301", and also the preparation of humanized forms of 0301. Those humanized forms of 0301 were produced using classical humanization techniques, i.e. by grafting of Kabat-defined murine CDRs into human heavy and light chain framework sequences, with some
20 of the human framework residues being potentially back-mutated to the correspondingly positioned 0301 murine residues. For reasons noted above, such humanized forms of 0301 described in WO2011/140249A2 are not ideal.

SUMMARY OF THE INVENTION

- 25 The present invention provides a number of anti-CSF1R antibodies and medical uses thereof.

- According to one aspect of the invention, there is provided an antibody molecule which specifically binds to human CSF1R, and optionally also to cynomolgus monkey CSF1R, or
30 an antigen-binding portion thereof, wherein the antibody molecule or antigen-binding portion comprises a heavy chain variable region with:

an HCDR1 having amino acids in sequence in the following order: G-Y/G-T-F-T/S-D/S-N/A/S/H/Y-Y/A-M/I-I/S (SEQ ID NO:13);

- an HCDR2 having amino acids in sequence in the following order: M or a conservative
35 substitution of M-G-D/G-I-N/I-P-Y/I-N/F-G-G/T-T/A-T/N-F/Y-N/A-Q-K-F-Q/K-G (SEQ ID NO:14); and

an HCDR3 having amino acids in sequence in the following order: E or a conservative substitution of E (such as D)-D/G/H/S/T/P/V/N/I/Y-P/T/N/E/L/A/D/S-Y/K/R/M/P-F/D/S/T/E/W/M/Y/L/Q/K/G/A/I-S/E/G/R-N/E/Q/G/H/M-L/H/S/Y-Y/W-V-M-D-Y (SEQ ID NO:15).

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In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequences GYTFTDNYMI (SEQ ID NO:16; 0301 murine/humanized antibody HCDR1 disclosed in WO2011/140249A2), DINPYNGGTTFNQKFKG (SEQ ID NO:17; 0301 murine/humanized antibody HCDR2 disclosed in WO2011/140249A2), and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence ESPYFSNLYVMDY (SEQ ID NO:18; 0301 murine/humanized antibody HCDR3 disclosed in WO2011/140249A2).

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The antibody molecule or antigen-binding portion may further comprise a light chain variable region with:

an LCDR1 having amino acids in sequence in the following order: R-A-S-Q-S-V-S/D/E-Y-D/E/Q-G-D/E-N-Y-L-N/A (SEQ ID NO:19);

an LCDR2 having amino acids in sequence in the following order: A/D-A-S-N/D-L/R-E/A-T (SEQ ID NO:20); and

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an LCDR3 having amino acids in sequence in the following order: Q/H/N-L/Q-S-N/S-E/Q/N-D/W-L-L/S-T (SEQ ID NO:21).

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In aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence KASQSVDYDGDNYMN (SEQ ID NO:22; 0301 murine/humanized antibody LCDR1 disclosed in WO2011/140249A2), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence AASNLES (SEQ ID NO:23; 0301 murine/humanized antibody LCDR2 disclosed in WO2011/140249A2) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence HLSNEDLST (SEQ ID NO:24; 0301 murine/humanized antibody LCDR3 disclosed in WO2011/140249A2).

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In some aspects, disclosed herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

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(a) the VH region amino acid sequence comprises HCDR1 of GYTFSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32) and HCDR3

of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38) and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(b) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60) and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(c) the VH region amino acid sequence comprises HCDR1 of GYTFSSAYMI (SEQ ID NO:57), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32) and HCDR3 of EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO:37), LCDR2 of AASDRAT (SEQ ID NO:60) and LCDR3 of QLSNEDLLT (SEQ ID NO:39); or

(d) the VH region amino acid sequence comprises HCDR1 of GYTFTSYMI (SEQ ID NO:41), HCDR2 of MGDINPYNGGTTYAQKFQG (SEQ ID NO:42) and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO:33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLA (SEQ ID NO:40), LCDR2 of AASNLAT (SEQ ID NO:43) and LCDR3 of QLSNEDLLT (SEQ ID NO:39).

In some aspects, disclosed herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

the VH region amino acid sequence comprises:

(a) HCDR1 of SEQ ID NO: 31, SEQ ID NO: 50, SEQ ID NO: 57 or SEQ ID NO: 41;
 (b) HCDR2 of SEQ ID NO: 32 or SEQ ID NO: 42; and
 (c) HCDR3 of SEQ ID NO: 33; and

the VL region amino acid sequence comprises:

(a') LCDR1 of SEQ ID NO: 37 or SEQ ID NO: 40;
 (b') LCDR2 of SEQ ID NO: 38, SEQ ID NO: 60 or SEQ ID NO: 43; and
 (c') LCDR3 of SEQ ID NO: 39.

In some aspects, disclosed herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises or consists of SEQ ID NO:352 and the VL region amino acid sequence comprises or consists of SEQ ID NO:351;

(b) the VH region amino acid sequence comprises or consists of SEQ ID NO:354 and the VL region amino acid sequence comprises or consists of SEQ ID NO:353;

(c) the VH region amino acid sequence comprises or consists of SEQ ID NO:356 and the VL region amino acid sequence comprises or consists of SEQ ID NO:355; or

5 (d) the VH region amino acid sequence comprises or consists of SEQ ID NO:358 and the VL region amino acid sequence comprises or consists of SEQ ID NO:357.

In some aspects, disclosed herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region comprising
10 HCDR1, HCDR2, and HCDR3 and a light chain variable (VL) region comprising LCDR1, LCDR2, and LCDR3, wherein

(a) the HCDR1 comprises the amino acid sequence G-X₁-T-F-X₂-X₃-X₄-X₅-X₆-X₇, wherein X₁ is Y or G, X₂ is T or S, X₃ is D or S, X₄ is N, A, S, H or Y, X₅ is Y or A, X₆ is M or I, and X₇ is I or S (SEQ ID NO: 25);

15 (b) the HCDR2 comprises the amino acid sequence X₁-G-X₂-I-X₃-P-X₄-X₅-G-X₆-X₇-X₈-X₉-X₁₀-Q-K-F-X₁₁-G, wherein X₁ is M or a conservative substitution of M, X₂ is D or G, X₃ is N or I, X₄ is Y or I, X₅ is N or F, X₆ is G or T, X₇ is T or A, X₈ is T or N, X₉ is F or Y, X₁₀ is N or A, and X₁₁ is Q or K (SEQ ID NO: 26);

(c) the HCDR3 comprises the amino acid sequence X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-V-
20 M-D-Y, wherein X₁ is E or a conservative substitution of E, X₂ is D, G, H, S, T, P, V, N, I, or Y, X₃ is P, T, N, E, L, A, D, or S, X₄ is Y, K, R, M, or P, X₅ is F, D, S, T, E, W, M, Y, L, Q, K, G, A, or I, X₆ is S, E, G, or R, X₇ is N, E, Q, G, H, or M, X₈ is L, H, S, or Y, and X₉ is Y or W (SEQ ID NO: 27);

(d) the LCDR1 comprises the amino acid sequence R-A-S-Q-S-V-X₁-Y-X₂-G-X₃-N-
25 Y-L-X₄, wherein X₁ is S, D, or E, X₂ is D, E, or Q, X₃ is D or E, and X₄ is N or A (SEQ ID NO: 28);

(e) the LCDR2 comprises the amino acid sequence X₁-A-S-X₂-X₃-X₄-T, wherein X₁ is A or D, X₂ is D or N, X₃ is L or R, and X₄ is E or A (SEQ ID NO: 29); and

(f) the LCDR3 comprises the amino acid sequence X₁-X₂-S-X₃-X₄-X₅-L-X₆-T, wherein
30 X₁ is Q, H, or N, X₂ is L or Q, X₃ is N or S, X₄ is E, Q, or N, X₅ is D or W, and X₆ is L or S (SEQ ID NO: 30).

In some aspects, the invention provides an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a
35 light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYQGENYLA (SEQ ID NO: 34), LCDR2 of DASNRAT (SEQ ID NO: 35),
5 and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(b) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38),
10 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(c) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLA (SEQ ID NO:40), LCDR2 of AASNRAT (SEQ ID NO:38),
15 and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(d) the VH region amino acid sequence comprises HCDR1 of GYTFTSYYYMI (SEQ ID NO:41), HCDR2 of MGDINPYNGGTTAQKFQG (SEQ ID NO: 42), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLA (SEQ ID NO:40), LCDR2 of AASNLAT (SEQ ID NO:43),
20 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(e) the VH region amino acid sequence comprises HCDR1 of GYTFTSYYYMI (SEQ ID NO:41), HCDR2 of MGDINPYNGGANFAQKFQG (SEQ ID NO:44), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38),
25 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(f) the VH region amino acid sequence comprises HCDR1 of GYTFTSYYYMI (SEQ ID NO:41), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38),
30 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(g) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYQGDNYLN (SEQ ID NO:45), LCDR2 of AASNRAT (SEQ ID NO:38),
35 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(h) the VH region amino acid sequence comprises HCDR1 of GYTFTSYYII (SEQ ID NO:46), HCDR2 of MGDINPYNGGATYAQKFQG (SEQ ID NO:47), and HCDR3 of EPPYFSNLYVMDY (SEQ ID NO:48); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYEGDNYLN (SEQ ID NO:49), LCDR2 of AASNRAT (SEQ ID NO:38),
5 and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(i) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGATYNQKFQG (SEQ ID NO:51), and HCDR3 of EPPYFSNLYVMDY (SEQ ID NO:48); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYEGENYLN (SEQ ID NO:52), LCDR2 of AASNRAT (SEQ ID NO:38),
10 and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(j) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGTTYAQKFQG (SEQ ID NO: 42), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYQGENYLN (SEQ ID NO:53), LCDR2 of AASNRAT (SEQ ID NO:38),
15 and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(k) the VH region amino acid sequence comprises HCDR1 of GYTFTSNYII (SEQ ID NO:54), HCDR2 of MGDINPYNGGTNYAQKFQG (SEQ ID NO:55), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYQGENYLN (SEQ ID NO:53), LCDR2 of AASNRAT (SEQ ID NO:38),
20 and LCDR3 of QLSSDLLT (SEQ ID NO:56);

(l) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38),
25 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(m) the VH region amino acid sequence comprises HCDR1 of GYTFSSAYMI (SEQ ID NO:57), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38),
30 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(n) the VH region amino acid sequence comprises HCDR1 of GYTFSSSYMI (SEQ ID NO:58), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38),
35 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(o) the VH region amino acid sequence comprises HCDR1 of GYTFSSHYMI (SEQ ID NO:59), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNDRAT (SEQ ID NO:38),
 5 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(p) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60),
 10 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(q) the VH region amino acid sequence comprises HCDR1 of GYTFSSAYMI (SEQ ID NO:57), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60),
 15 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(r) the VH region amino acid sequence comprises HCDR1 of GYTFSSSYMI (SEQ ID NO:58), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60),
 20 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39); or

(s) the VH region amino acid sequence comprises HCDR1 of GYTFSSHYMI (SEQ ID NO:59), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60),
 25 and LCDR3 of QLSNEDLLT (SEQ ID NO: 39).

Further provided herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH region amino acid sequence comprises SEQ ID NO:1, SEQ ID
 30 NO:2, SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; or SEQ ID NO:8; and the VL region amino acid sequence comprises SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11 or SEQ ID NO:12.

Additionally provided herein is an anti-CSF1R antibody or an antigen-binding portion
 35 thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

- (a) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (b) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:10;
- 5 (c) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:11;
- (d) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:12;
- (e) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:9;
- 10 (f) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:10;
- (g) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:11;
- 15 (h) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:12;
- (i) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (j) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:10;
- 20 (k) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:11;
- (l) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:12;
- 25 (m) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (n) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:10;
- (o) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:11;
- 30 (p) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:12;
- (q) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:9;
- 35 (r) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:10;

- (s) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:11;
- (t) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:12;
- 5 (u) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (v) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL region amino acid sequence comprises SEQ ID NO:10;
- (w) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL
- 10 region amino acid sequence comprises SEQ ID NO:11;
- (x) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL region amino acid sequence comprises SEQ ID NO:12;
- (y) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL region amino acid sequence comprises SEQ ID NO:9;
- 15 (z) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL region amino acid sequence comprises SEQ ID NO:10;
- (aa) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL region amino acid sequence comprises SEQ ID NO:11;
- (bb) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL
- 20 region amino acid sequence comprises SEQ ID NO:12;
- (cc) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (dd) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:10;
- 25 (ee) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:11; or
- (ff) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:12.
- 30 Also provided according to the invention is an immunoconjugate comprising the antibody molecule or antigen-binding portion thereof as defined herein linked, fused or conjugated to a therapeutic agent.
- In another aspect the invention provides a nucleic acid molecule encoding the antibody
- 35 molecule or antigen-binding portion thereof as defined herein.

Further provided is a vector comprising the nucleic acid molecule of the invention.

Also provided is a host cell comprising the nucleic acid molecule or the vector of the invention as defined herein.

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In a further aspect there is provided a method of producing an anti-CSF1R antibody and/or an antigen-binding portion thereof, comprising culturing the host cell of the invention under conditions that result in expression and/or production of the antibody and/or the antigen-binding portion thereof, and isolating the antibody and/or the antigen-binding portion thereof

10 from the host cell or culture.

In another aspect of the invention there is provided a pharmaceutical composition comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein.

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Further provided is a method for enhancing an immune response in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

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In a further aspect there is provided a method for treating or preventing cancer in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

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Further provided herein is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein, for use as a medicament. Also provided is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein, for use in the treatment of cancer.

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In another aspect the invention provides the antibody molecule, or antigen-binding portion thereof, or the immunoconjugate, or the nucleic acid molecule, or the vector for use, or the method of treatment of the invention as defined herein, for separate, sequential or
5 simultaneous use in a combination combined with a second therapeutic agent, for example an anti-cancer agent.

In a further aspect there is provided the use of an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or an immunoconjugate of the invention
10 as defined herein, or a nucleic acid molecule of the invention as defined herein, or a vector of the invention as defined herein, or a pharmaceutical composition of the invention as defined herein, in the manufacture of a medicament for the treatment of cancer.

The invention also provides a method for treating or preventing an infectious disease in a
15 subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

20 The infectious disease may be selected in all aspects from the group consisting of: viral, bacterial, fungal or parasitic.

Also provided is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein,
25 or the vector as defined herein, or the pharmaceutical composition as defined herein, for use in the treatment of an infectious disease.

Further provided is the use of an antibody molecule or antigen-binding portion thereof as defined herein, or an immunoconjugate as defined herein, or a nucleic acid molecule as
30 defined herein, or a vector as defined herein, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for the treatment of an infectious disease.

The invention also provides a method for treating or preventing an infectious disease in a
35 subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the

nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

The invention also provides a method of producing an antibody molecule which specifically
5 binds to human CSF1R and optionally also to cynomolgus and monkey CSF1R, or an antigen-binding portion thereof, comprising the steps of:

- (1) grafting anti-CSF1R CDRs from a non-human source into a human v-domain framework to produce a humanized anti-CSF1R antibody molecule or antigen-binding portion thereof;
- (2) generating a library of clones of the humanized anti-CSF1R antibody molecule or antigen-
10 binding portion thereof comprising one or more mutations in the CDRs;
- (3) screening the library for binding to human CSF1R and optionally also to cynomolgus and monkey CSF1R;
- (4) selecting clones from the screening step (3) having binding specificity to human CSF1R and optionally also to cynomolgus and rhesus monkey CSF1R; and
- 15 (5) producing an antibody molecule which specifically binds to human CSF1R and optionally also to cynomolgus and rhesus monkey CSF1R, or an antigen-binding portion thereof from clones selected from step (4).

The method may comprise a further step of producing additional clones based on the clones
20 selected in step (4), for example based on further exploratory mutagenesis at specific positions in the CDRs of the clones selected in step (4), to enhance humanization and/or minimise human T cell epitope content and/or improve manufacturing properties in the antibody molecule or antigen-binding portion thereof produced in step (5).

25 BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Direct binding ELISA of library-derived anti-CSF1R Fabs against human and cyno CSF1R-Fc proteins. Clones were derived from multiple phage selection branches where phage populations were selected on biotinylated human, or cynomolgus monkey CSF1R proteins in each round. Standard selections are numbered R2-R4. 'Hammer-Hug'
30 rounds are numbered R2-H and R3-H. After each round of selection, library-derived clones (black circles) were screened as periplasmically-expressed Fab proteins, against both human (h) and cyno (c) CSF1R. Mean $\pm$ SD values in each round are represented in black bars.

FIG. 2A – FIG. 2B. Analysis of CDR residue tolerance for mutation to germline. A plot
35 of murine amino acid retention frequencies in the CDRs of the ELISA-positive population of 598 unique scFv clones is shown for V_L (SEQ ID NOs: 22, 23 and 24) (FIG. 2A) and V_H (SEQ

ID NOs: 16, 17 and 18) (FIG. 2B) domains, respectively. Only those residues targeted for human/murine residue mutagenesis are plotted, other than in the HCDR3. CDR residues noted in parentheses on the X-axes were identical to those found in the human germlines used for grafting (IGKV3-11 and IGHV1-69). In both plots the dashed line in grey at 75% represents the cut-off for tolerance of murine residue replacement by human germline.

FIG. 3A – FIG. 3B. Direct titration ELISA for library-derived and designer IgG4(S228P) clones binding to human and cyno CSF1R-Fc proteins. mu0301, hu0301, library-derived and designer clones in human IgG4(S228P) format were titrated (in nM) in a direct binding ELISA against human (FIG. 3A) and cyno (FIG. 3B) CSF1R-Fc proteins.

FIG. 4. Epitope competition analysis of IgG4 proteins in Alphascreen.

Anti-CSF1R IgG4(S228P) clones were applied in an epitope competition assay using Alphascreen technology. In this assay, library-derived (A) and designer (B) IgGs were analysed for their relative affinities and retention of the parental mu0301 epitope by competing for mu0301 IgG4(S228P) binding to human CSF1R protein, in solution. All clones analysed showed strong, concentration-dependent neutralisation of mu0301 binding to CSF1R.

FIG. 5A – FIG. 5B. Flow cytometric binding to human and rhesus CSF1R+ HEK-293 cells. mu0301, hu0301, lead library-derived and designer IgGs were examined for specific binding on HEK-293 cells expressing human (FIG. 5A) and rhesus (FIG. 5B) CSF1R.

Concentration-dependent binding was observed against human CSF1R for all clones, with binding curves that overlap or improve over the hu0301 clone.

FIG. 6A – FIG. 6B. Direct titration ELISA for designer, deimmunized IgG4(S228P) clones binding to human and cyno CSF1R-Fc proteins. mu0301, hu0301, isotype and designer clones in human IgG4(S228P) format were titrated (in nM) in a direct binding ELISA against human (FIG. 6A) and cyno (FIG. 6B) CSF1R-Fc proteins.

FIG. 7. Flow cytometric binding to human CSF1R+ HEK-293 cells. mu0301, hu0301, isotype and designer clones in human IgG4(S228P) format were examined for specific binding on HEK-293 cells expressing human CSF1R. Concentration-dependent binding was observed against human CSF1R for all clones, with binding curves that overlap with the hu0301 clone.

FIG. 8. Development risk ELISAs. mu0301, hu0301, isotype and designer clones in human IgG4(S228P) format were examined for nonspecific binding to the negatively charged biomolecules insulin and double-stranded DNA (dsDNA). All lead clones demonstrated binding scores of 1.0, significantly lower than either of the negative control IgG1 Ustekinumab and Bevacizumab analogs. Strong off-target binding to human insulin or

dsDNA, as observed for Bococizumab and Briakinumab analogues, has been shown to be a high-risk indicator of poor pharmacokinetics of therapeutic antibodies.

FIG. 9A – FIG. 9C. Effect of anti-CSF1R antibodies on primary human monocyte proliferation.

5 hu0301, isotype and lead clones in human IgG4(S228P) format were examined for the potency of inhibition of human monocyte proliferation (under MCSF stimulation), using monocyte populations from 3 separate human donors. All clones examined were found to have fully overlapping curves across all 3 donors, indicating highly similar biological potency.

FIG. 10. Differential Scanning Calorimetry (DSC) of IgGs. DSC assay data for the following antibodies in IgG4(S228P) form: (mAb-1) hu0301, (mAb-2) MH5, (mAb-3) 30E06, (mAb-4) MH12, and (mAb-5) MH16.

FIG. 11A – FIG. 11E. Reverse Phase Chromatography analyses of tryptic peptides from IgGs before and after forced oxidation. Reverse phase chromatograms for antibodies in IgG4(S228P) form: hu0301 (FIG. 11A) exhibited 8 significant peptide changes post-oxidation. MH5 (FIG. 11B) exhibited 3, 30E06 (FIG. 11C) exhibited 4, MH12 (FIG. 11D) exhibited 4 and MH16 (FIG. 11E) exhibited 5. In each panel chromatograms before (-H₂O₂, upper) and after oxidation (+H₂O₂, lower) are shown. Arrows in each panel indicate peaks representing modified peptides (gained, reduced or lost after oxidation).

FIG. 12. HIC chromatograms of IgGs. A panel of clinical monoclonal antibodies (Adalimumab, Brentuximab, Cetuximab, Golimumab and Trastuzumab) and antibodies in IgG4(S228P) form: (mAb-1) hu0301, (mAb-2) MH5, (mAb-3) 30E06, (mAb-4) MH12, and (mAb-5) MH16, were analysed by HIC. All antibodies eluted between 3.5 and 10.5 minutes (left to right).

25 DETAILED DESCRIPTION OF THE INVENTION

According to a first aspect of the invention, there is provided an antibody molecule which specifically binds to human CSF1R and optionally also to cynomolgus monkey CSF1R, or an antigen-binding portion thereof, wherein the antibody molecule or antigen-binding portion comprises a heavy chain variable region with:

30 an HCDR1 having amino acids in sequence in the following order: G-Y/G-T-F-T/S-D/S-N/A/S/H/Y-Y/A-M/I-I/S (SEQ ID NO:13);

an HCDR2 having amino acids in sequence in the following order: M or a conservative substitution of M-G-D/G-I-N/I-P-Y/I-N/F-G-G/T-T/A-T/N-F/Y-N/A-Q-K-F-Q/K-G (SEQ ID NO:14); and

35 an HCDR3 having amino acids in sequence in the following order: E or a conservative substitution of E (such as D)-D/G/H/S/T/P/V/N/I/Y-P/T/N/E/L/A/D/S-Y/K/R/M/P-

F/D/S/T/E/W/M/Y/L/Q/K/G/A/I-S/E/G/R-N/E/Q/G/H/M-L/H/S/Y-Y/W-V-M-D-Y (SEQ ID NO:15).

In some aspects an anti-CSF1R antibody or antigen-binding portion provided herein specifically binds to a CSF1R protein comprising or consisting of SEQ ID NO:365 or 366. In some aspects an anti-CSF1R antibody or antigen-binding portion provided herein specifically binds to a CSF1R protein having an amino acid sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:365 or 366.

In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequences GYTFTDNYMI (SEQ ID NO:16; 0301 murine/humanized antibody HCDR1 disclosed in WO2011/140249A2), DINPYNGGTTFNQKFKG (SEQ ID NO:17; 0301 murine/humanized antibody HCDR2 disclosed in WO2011/140249A2), and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence ESPYFSNLYVMDY (SEQ ID NO:18; 0301 murine/humanized antibody HCDR3 disclosed in WO2011/140249A2).

The antibody molecule or antigen-binding portion may further comprise a light chain variable region with:
 an LCDR1 having amino acids in sequence in the following order: R-A-S-Q-S-V-S/D/E-Y-D/E/Q-G-D/E-N-Y-L-N/A (SEQ ID NO:19);
 an LCDR2 having amino acids in sequence in the following order: A/D-A-S-N/D-L/R-E/A-T (SEQ ID NO:20); and
 an LCDR3 having amino acids in sequence in the following order: Q/H/N-L/Q-S-N/S-E/Q/N-D/W-L-L/S-T (SEQ ID NO:21).

In aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence KASQSVDYDGDNYMN (SEQ ID NO:22; 0301 murine/humanized antibody LCDR1 disclosed in WO2011/140249A2), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence AASNLES (SEQ ID NO:23; 0301 murine/humanized antibody LCDR2 disclosed in WO2011/140249A2) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence HLSNEDLST (SEQ ID NO:24; 0301 murine/humanized antibody LCDR3 disclosed in WO2011/140249A2).

In some aspects, disclosed herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region comprising HCDR1, HCDR2, and HCDR3 and a light chain variable (VL) region comprising LCDR1, LCDR2, and LCDR3, wherein

(a) the HCDR1 comprises the amino acid sequence G-X₁-T-F-X₂-X₃-X₄-X₅-X₆-X₇, wherein X₁ is Y or G, X₂ is T or S, X₃ is D or S, X₄ is N, A, S, H or Y, X₅ is Y or A, X₆ is M or I, and X₇ is I or S (SEQ ID NO: 25);

(b) the HCDR2 comprises the amino acid sequence X₁-G-X₂-I-X₃-P-X₄-X₅-G-X₆-X₇-X₈-X₉-X₁₀-Q-K-F-X₁₁-G, wherein X₁ is M or a conservative substitution of M, X₂ is D or G, X₃ is N or I, X₄ is Y or I, X₅ is N or F, X₆ is G or T, X₇ is T or A, X₈ is T or N, X₉ is F or Y, X₁₀ is N or A, and X₁₁ is Q or K (SEQ ID NO: 26);

(c) the HCDR3 comprises the amino acid sequence X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-V-M-D-Y, wherein X₁ is E or a conservative substitution of E (for example, D), X₂ is D, G, H, S, T, P, V, N, I, or Y, X₃ is P, T, N, E, L, A, D, or S, X₄ is Y, K, R, M, or P, X₅ is F, D, S, T, E, W, M, Y, L, Q, K, G, A, or I, X₆ is S, E, G, or R, X₇ is N, E, Q, G, H, or M, X₈ is L, H, S, or Y, and X₉ is Y or W (SEQ ID NO: 27);

(d) the LCDR1 comprises the amino acid sequence R-A-S-Q-S-V-X₁-Y-X₂-G-X₃-N-Y-L-X₄, wherein X₁ is S, D, or E, X₂ is D, E, or Q, X₃ is D or E, and X₄ is N or A (SEQ ID NO: 28);

(e) the LCDR2 comprises the amino acid sequence X₁-A-S-X₂-X₃-X₄-T, wherein X₁ is A or D, X₂ is D or N, X₃ is L or R, and X₄ is E or A (SEQ ID NO: 29); and

(f) the LCDR3 comprises the amino acid sequence X₁-X₂-S-X₃-X₄-X₅-L-X₆-T, wherein X₁ is Q, H, or N, X₂ is L or Q, X₃ is N or S, X₄ is E, Q, or N, X₅ is D or W, and X₆ is L or S (SEQ ID NO: 30).

In some aspects, disclosed herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region comprising, in amino-terminal to carboxyl-terminal order, FR1-HCDR1-FR2-HCDR2-FR3-HCDR3-FR4 and a light chain variable (VL) region comprising, in amino-terminal to carboxyl-terminal order, FR1-LCDR1-FR2-LCDR2-FR3-LCDR3-FR4, wherein the HCDR1 is SEQ ID NO:25, the HCDR2 is SEQ ID NO:26, the HCDR3 is SEQ ID NO:27, the LCDR1 is SEQ ID NO:28, the LCDR2 is SEQ ID NO:29 and the LCDR3 is SEQ ID NO:30, wherein the heavy chain FR1, FR2, FR3 and FR4 amino acid sequences are the heavy chain FR1, FR2, FR3 and FR4 amino acid sequences in SEQ ID NO: 105 (see Table 2) and wherein the light chain

FR1, FR2, FR3 and FR4 amino acid sequences are the light chain FR1, FR2, FR3 and FR4 amino acid sequences in SEQ ID NO: 108 (see Table 2).

As elaborated herein, the present inventors have succeeded for the first time in generating
5 a number of optimized anti-CSF1R antibody molecules using CDR sequences derived from the murine anti-CSF1R antibody 0301 disclosed in WO2011/140249A2. In embodiments of the present invention, these antibody molecules have been selected to have highly similar binding specificity and affinity to both human CSF1R as well as cynomolgus and rhesus monkey CSF1R (to facilitate maximally accurate primate toxicology and pk studies). Further
10 refining of the optimized antibody molecules as described herein has provided improved binding to the human and cynomolgus monkey orthologues of CSF1R, improved potency in neutralisation of CSF1R signalling, improved variable domain stability, high expression yields, and/or reduced immunogenicity potential.

15 In some aspects, optimized anti-CSF1R antibody molecules of the present invention do not necessarily have the maximum number of human germline substitutions at corresponding murine CDR or other (such as framework) amino acid positions. As elaborated in the experimental section below, we have found that “maximally humanized” antibody molecules are not necessary “maximally optimized” in terms of anti-CSF1R binding characteristics
20 and/or other desirable features.

The present invention encompasses modifications to the amino acid sequence of the antibody molecule or antigen-binding portion thereof as defined herein. For example, the invention includes antibody molecules and corresponding antigen-binding portions thereof
25 comprising functionally equivalent variable regions and CDRs which do not significantly affect their properties as well as variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence may be mutated to obtain an antibody with the desired binding affinity to CSF1R. Insertions which include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or
30 more residues, as well as intrasequence insertions of single or multiple amino acid residues, are envisaged. Examples of terminal insertions include an antibody molecule with an N-terminal methionyl residue or the antibody molecule fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the half-life of the antibody in the blood
35 circulation.

The antibody molecule or antigen-binding portion of the invention may include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. The antibody molecule or antigen-binding portion of the invention may be mutated to alter such post-translational modifications, for example by adding, removing or replacing one or more amino acid residues to form or remove a glycosylation site.

The antibody molecule or antigen-binding portion of the invention may be modified for example by amino acid substitution to remove potential proteolytic sites in the antibody.

10

In the antibody molecule or antigen-binding portion thereof, the HCDR1 may have the amino acid sequence: G-Y/G-T-F-T/S-D/S-N/A/S/H/Y-Y/A-M/I-I (SEQ ID NO:61); the HCDR2 may have the amino acid sequence: M-G-D-I-N/I-P-Y-N/F-G-G/T-T/A-T/N-F/Y-N/A-Q-K-F-Q-G (SEQ ID NO:62); and the HCDR3 may have the amino acid sequence: E/D-D/G/H/S/T/P/V/N/I/Y-P/T/N/E/L/A/D/S-Y/K/R/M/P-F/D/S/T/E/W/M/Y/L/Q/K/G/A/I-S/E/G/R-N/E/Q/G/H/M-L/H/S/Y-Y/W-V-M-D-Y (SEQ ID NO:63).

For example, the HCDR1 may have the amino acid sequence: G-Y-T-F-T/S-S-N/A/S/H/Y-Y-M/I-I/S (SEQ ID NO:64); the HCDR2 may have the amino acid sequence: M-G-D-I-N-P-Y-N-G-G/T-T/A-T/N-Y-N/A-Q-K-F-Q-G (SEQ ID NO:65); and the HCDR3 may have the amino acid sequence: E-G/P/D-P-Y-F-S-N-L-Y-V-M-D-Y (SEQ ID NO:66).

In the antibody molecule or antigen-binding portion thereof, the LCDR1 may have the amino acid sequence: R-A-S-Q-S-V-S/D/E-Y-D/E/Q-G-D/E-N-Y-L-N/A (SEQ ID NO:19); the LCDR2 may have the amino acid sequence A/D-A-S-D/N-L/R-E/A-T (SEQ ID NO:67); and the LCDR3 may have the amino acid sequence: Q/H/N-L/Q-S-N/S-E/Q/N-D-L-L-T (SEQ ID NO:68).

For example, the LCDR1 may have the amino acid sequence: R-A-S-Q-S-V-S/E-Y-E/Q-G-D/E-N-Y-L-N/A (SEQ ID NO:69); the LCDR2 may have the amino acid sequence A/D-A-S-D/N-L/R-A-T (SEQ ID NO:70); and the LCDR3 may have the amino acid sequence: Q-L-S-N-E/Q-D-L-L-T (SEQ ID NO:71).

In specific embodiments of the invention, the antibody molecule or antigen-binding portion may comprise:

(A) the amino acid sequences

GYTFSSYYMI (HCDR1; SEQ ID NO: 31), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYQGENYLA (LCDR1; SEQ ID NO:34), DASNRAT (LCDR2; SEQ ID NO: 35) and QLSNQDLLT (LCDR3; SEQ ID NO:36) [Clone MH4]; or

5 (B) the amino acid sequences

GYTFSSYYMI (HCDR1; SEQ ID NO: 31), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLN (LCDR1; SEQ ID NO: 37), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone MH5];

10 (C) the amino acid sequences

GYTFSSYYMI (HCDR1; SEQ ID NO: 31), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLA (LCDR1; SEQ ID NO:40), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNQDLLT (LCDR3; SEQ ID NO:36) [Clone MH6];

15 (D) the amino acid sequences

GYTFTSYMI (HCDR1; SEQ ID NO: 41), MGDINPYNGGTTAQKFQG (HCDR2; SEQ ID NO:42), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLA (LCDR1; SEQ ID NO:40), AASNLAT (LCDR2; SEQ ID NO:43) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone 30E6];

20 (E) the amino acid sequences

GYTFTSYMI (HCDR1; SEQ ID NO: 41), MGDINPYNGGANFAQKFQG (HCDR2; SEQ ID NO:44), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLN (LCDR1; SEQ ID NO: 37), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone 29D10];

25 (F) the amino acid sequences

GYTFTSYMI (HCDR1; SEQ ID NO: 41), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLN (LCDR1; SEQ ID NO: 37), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone 29H09];

30 (G) the amino acid sequences

GYTFSSYYMI (HCDR1; SEQ ID NO: 31), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVEYQGDNYLN (LCDR1; SEQ ID NO:45), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone 26B07];

35 (H) the amino acid sequences

- GYTFTSYII (HCDR1; SEQ ID NO:46), MGDINPYNGGATYAQKFQG (HCDR2; SEQ ID NO:47), EPPYFSNLYVMDY (HCDR3; SEQ ID NO:48), RASQSVEYEGDNYLN (LCDR1; SEQ ID NO:49), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNQDLLT (LCDR3; SEQ ID NO:36) [Clone 29B07];
- 5 (I) the amino acid sequences
GYTFSSNYMI (HCDR1; SEQ ID NO:50), MGDINPYNGGATYNQKFQG (HCDR2; SEQ ID NO:51), EPPYFSNLYVMDY (HCDR3; SEQ ID NO:48), RASQSVEYEGENYLN (LCDR1; SEQ ID NO:52), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNQDLLT (LCDR3; SEQ ID NO:36) [Clone 29A03];
- 10 (J) the amino acid sequences
GYTFSSYMI (HCDR1; SEQ ID NO: 31), MGDINPYNGGTTA QKFQG (HCDR2; SEQ ID NO:42), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVEYQGENYLN (LCDR1; SEQ ID NO:53), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNQDLLT (LCDR3; SEQ ID NO:36) [Clone 30D02];
- 15 (K) the amino acid sequences
GYTFTSNYII (HCDR1; SEQ ID NO:54), MGDINPYNGGTNYAQKFQG (HCDR2; SEQ ID NO:55), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVEYQGENYLN (LCDR1; SEQ ID NO:53), AASNRAT (LCDR2; SEQ ID NO:38) and QLSSEDLLT (LCDR3; SEQ ID NO:56) [Clone 30G02];
- 20 (L) the amino acid sequences
GYTFSSNYMI (HCDR1; SEQ ID NO:50), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLN (LCDR1; SEQ ID NO: 37), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone MH10, MH11, MH13, MH26, MH27, MH29];
- 25 (M) the amino acid sequences
GYTFSSAYMI (HCDR1; SEQ ID NO:57), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLN (LCDR1; SEQ ID NO: 37), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone MH14, MH15, MH17, MH30, MH31, MH33];
- 30 (N) the amino acid sequences
GYTFSSSYMI (HCDR1; SEQ ID NO:58), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLN (LCDR1; SEQ ID NO: 37), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone MH18, MH19, MH21, MH34, MH35, MH37];
- 35 (O) the amino acid sequences

GYTFSSHIMI (HCDR1; SEQ ID NO:59), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLN (LCDR1; SEQ ID NO: 37), AASNRAT (LCDR2; SEQ ID NO:38) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone MH22, MH23, MH25, MH38, MH39, MH41];

5 (P) the amino acid sequences

GYTFSSNYMI (HCDR1; SEQ ID NO:50), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLN (LCDR1; SEQ ID NO: 37), AASDRAT (LCDR2; SEQ ID NO:60) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone MH12, MH28];

10 (Q) the amino acid sequences

GYTFSSAYMI (HCDR1; SEQ ID NO:57), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLN (LCDR1; SEQ ID NO: 37), AASDRAT (LCDR2; SEQ ID NO:60) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone MH16, MH32];

15 (R) the amino acid sequences

GYTFSSSYMI (HCDR1; SEQ ID NO:58), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLN (LCDR1; SEQ ID NO: 37), AASDRAT (LCDR2; SEQ ID NO:60) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone MH20, MH36]; or

20 (S) the amino acid sequences

GYTFSSHIMI (HCDR1; SEQ ID NO:59), MGDINPYNGGANYAQKFQG (HCDR2; SEQ ID NO: 32), EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33), RASQSVSYEGENYLN (LCDR1; SEQ ID NO: 37), AASDRAT (LCDR2; SEQ ID NO:60) and QLSNEDLLT (LCDR3; SEQ ID NO: 39) [Clone MH24, MH40].

25

In some aspects, the invention provides an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GYTFSSYIMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYQGENYLA (SEQ ID NO: 34), LCDR2 of DASNRAT (SEQ ID NO: 35), and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(b) the VH region amino acid sequence comprises HCDR1 of GYTFSSYIMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises

LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(c) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLA (SEQ ID NO:40), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(d) the VH region amino acid sequence comprises HCDR1 of GYTFTSYYYMI (SEQ ID NO:41), HCDR2 of MGDINPYNGGTTYAQKFQG (SEQ ID NO: 42), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLA (SEQ ID NO:40), LCDR2 of AASNLAT (SEQ ID NO:43), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(e) the VH region amino acid sequence comprises HCDR1 of GYTFTSYYYMI (SEQ ID NO:41), HCDR2 of MGDINPYNGGANFAQKFQG (SEQ ID NO:44), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(f) the VH region amino acid sequence comprises HCDR1 of GYTFTSYYYMI (SEQ ID NO:41), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(g) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYQGDNYLN (SEQ ID NO:45), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(h) the VH region amino acid sequence comprises HCDR1 of GYTFTSYYYII (SEQ ID NO:46), HCDR2 of MGDINPYNGGATYAQKFQG (SEQ ID NO:47), and HCDR3 of EPPYFSNLYVMDY (SEQ ID NO:48); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYEGDNYLN (SEQ ID NO:49), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(i) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGATYNQKFQG (SEQ ID NO:51), and HCDR3 of EPPYFSNLYVMDY (SEQ ID NO:48); and the VL region amino acid sequence comprises

LCDR1 of RASQSVEYEGENYLN (SEQ ID NO:52), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(j) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGTTYAQKFQG (SEQ ID NO: 42), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYQGENYLN (SEQ ID NO:53), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(k) the VH region amino acid sequence comprises HCDR1 of GYTFTSNYII (SEQ ID NO:54), HCDR2 of MGDINPYNGGTNYAQKFQG (SEQ ID NO:55), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYQGENYLN (SEQ ID NO:53), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSSDLLT (SEQ ID NO:56);

(l) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(m) the VH region amino acid sequence comprises HCDR1 of GYTFSSAYMI (SEQ ID NO:57), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(n) the VH region amino acid sequence comprises HCDR1 of GYTFSSSYMI (SEQ ID NO:58), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(o) the VH region amino acid sequence comprises HCDR1 of GYTFSSHYMI (SEQ ID NO:59), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(p) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises

LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(q) the VH region amino acid sequence comprises HCDR1 of GYTFSSAYMI (SEQ ID NO:57), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(r) the VH region amino acid sequence comprises HCDR1 of GYTFSSSYMI (SEQ ID NO:58), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39); or

(s) the VH region amino acid sequence comprises HCDR1 of GYTFSSHYMI (SEQ ID NO:59), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60), and LCDR3 of QLSNEDLLT (SEQ ID NO:39).

In some embodiments, an anti-CSF1R antibody or antigen-binding portion thereof comprises the six CDRs of any one of clones 29D10, 29B07, 30C11, 26B07, 29A03, 29E11, 30G02, 30E06, 29H09, 30D02, MH1, MH2, MH3, MH4, MH5, MH6, MH7, MH8 or MH9 (see Table 4) and framework regions (FRs) comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 22, 25, 27, 30, 33 or 35 immunogenicity-reducing mutations compared to sequences of human germline v-domain FRs. The immunogenicity-reducing mutations may be present in the VH framework regions, the VL framework regions, or both the VH and the VL framework regions. For example, an immunogenicity-reducing mutation may be present in the VH FR1, VH FR2, VH FR3, VH FR4, VL FR1, VL FR2, VL FR3, VL FR4, or any combination of these FRs. In some embodiments, the immunogenicity-reducing mutation is an amino acid substitution, deletion or insertion.

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In some aspects, disclosed herein is anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH region comprises any one of the VH region amino acid sequences in Table 11 or 16 and the VL region comprises any one of the VL region amino acid sequences in Table 11 or 16.

35

In some embodiments, provided herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH region amino acid sequence comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; or SEQ ID NO:8. Further provided herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VL region amino acid sequence comprises SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11 or SEQ ID NO:12. Also provided herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH region amino acid sequence comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; or SEQ ID NO:8; and the VL region amino acid sequence comprises SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11 or SEQ ID NO:12.

- In some embodiments, provided herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein
- (a) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:9;
 - (b) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:10;
 - (c) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:11;
 - (d) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:12;
 - (e) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:9;
 - (f) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:10;
 - (g) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:11;
 - (h) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:12;
 - (i) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:9;

- (j) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:10;
- (k) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:11;
- 5 (l) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:12;
- (m) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (n) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:10;
- 10 (o) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:11;
- (p) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:12;
- 15 (q) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (r) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:10;
- (s) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:11;
- 20 (t) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:12;
- (u) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL region amino acid sequence comprises SEQ ID NO:9;
- 25 (v) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL region amino acid sequence comprises SEQ ID NO:10;
- (w) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL region amino acid sequence comprises SEQ ID NO:11;
- (x) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL region amino acid sequence comprises SEQ ID NO:12;
- 30 (y) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (z) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL region amino acid sequence comprises SEQ ID NO:10;
- 35 (aa) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL region amino acid sequence comprises SEQ ID NO:11;

(bb) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL region amino acid sequence comprises SEQ ID NO:12;

(cc) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:9;

5 (dd) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:10;

(ee) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:11; or

10 (ff) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:12.

In some aspects, disclosed herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

15 (a) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:352 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at
20 least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:351;

(b) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ
25 ID NO:354 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:353;

(c) the VH region amino acid sequence is at least about 90%, at least about 91%,
30 at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:356 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to
35 SEQ ID NO:355; or

(d) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:358 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:357. In some aspects, the CDR amino acid sequences of an anti-CSF1R antibody are 100% identical to the CDR amino acid sequences in the recited sequences while the FR amino acid sequences are less than 100% identical to the FR amino acid sequences in the recited sequences.

In some aspects, the antibody or antigen-binding portion as defined herein may be isolated.

The antibody molecule or antigen-binding portion as defined herein may cross-compete for binding to CSF1R with an antibody or antigen-binding portion thereof comprising the sets of CDRs disclosed herein. In some embodiments, the invention provides an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody or antigen-binding portion cross-competes for binding to CSF1R with an antibody or antigen-binding portion whose sequences are provided herein; and (a) comprises fully germline human framework amino acid sequences; (b) binds specifically to human CSF1R, cynomolgus CSF1R, and rhesus CSF1R; (c) binds to monomeric cynomolgus CSF1R with a K_D lower than 0.66 nM and to human CSF1R with a K_D lower than 0.21 nM; and/or (d) binds to a functionally identical epitope on cynomolgus CSF1R and rhesus CSF1R. In some embodiments, the invention provides an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody or antigen-binding portion cross-competes for binding to CSF1R with an antibody or antigen-binding portion whose sequences are provided herein; and (a) comprises fully germline human framework amino acid sequences; (b) comprises the IGHV1-69*01 allele germline framework regions in the VH domain; (c) does not contain an isomerisation risk motif in the LCDR1 sequence; (d) binds specifically to human CSF1R, cynomolgus CSF1R, and rhesus CSF1R; (e) binds to human CSF1R with a K_D that is within 3-fold of said antibody's or antigen-binding portion's K_D for binding to cynomolgus CSF1R; (f) does not comprise a human T cell epitope sequence in the Framework 3/LCDR3 region of the VL domain; and/or (g) does not comprise a human T cell epitope sequence in the HCDR-2/Framework 3 region of the VH domain; and/or (h) does not comprise a human T cell epitope sequence in the HCDR1/Framework 2 region of the VH domain; and/or (i) does not comprise a human T cell epitope sequence in the

- HCDR3/Framework 4 region of the VH domain; and/or (j) does not comprise a human T cell epitope sequence in the Framework 2/LCDR2 region of the VL domain; and/or (k) exhibits minimal or no binding to insulin (e.g., human insulin) and/or double-stranded DNA (dsDNA); and/or (l) exhibits reduced susceptibility to oxidative damage compared to antibody hu0301, when in IgG4(S228P) antibody format; and/or (m) exhibits improved thermal stability in the Fab domain compared to antibody hu0301, when in IgG4(S228P) antibody format; and/or (n) exhibits reduced hydrophobic species heterogeneity compared to antibody hu0301, when in IgG4(S228P) antibody format.
- 10 In some embodiments, an anti-CSF1R antibody or an antigen-binding portion of the invention binds to monomeric cynomolgus CSF1R with a KD lower than 2 nM and to human CSF1R with a KD lower than 2 nM. In some embodiments, an anti-CSF1R antibody or an antigen-binding portion of the invention binds to monomeric cynomolgus CSF1R with a KD lower than 1 nM and to human CSF1R with a KD lower than 1 nM.
- 15 In some embodiments, a KD value of an antibody or antigen-binding portion may be determined by Biacore analysis. In some embodiments, an EC50 value of an antibody or antigen-binding portion may be determined by flow cytometric staining of CSF1R expressing cells (e.g., HEK cells).
- 20 The terms "cross-compete", "cross-competition", "cross-block", "cross-blocked" and "cross-blocking" are used interchangeably herein to mean the ability of an antibody or portion thereof to interfere with the binding directly or indirectly through allosteric modulation of the anti-CSF1R antibodies of the invention to the target CSF1R (e.g., human CSF1R). The extent to which an antibody or portion thereof is able to interfere with the binding of another to the target, and therefore whether it can be said to cross-block or cross-compete according to the invention, can be determined using competition binding assays. One example of a binding competition assay is Homogeneous Time Resolved Fluorescence (HTRF). One particularly suitable quantitative cross-competition assay uses a FACS- or an
- 25 AlphaScreen-based approach to measure competition between the labelled (e.g. His tagged, biotinylated or radioactive labelled) antibody or portion thereof and the other antibody or portion thereof in terms of their binding to the target. In general, a cross-competing antibody or portion thereof is, for example, one which will bind to the target in the cross-competition assay such that, during the assay and in the presence of a second
- 30 antibody or portion thereof, the recorded displacement of the immunoglobulin single variable domain or polypeptide according to the invention is up to 100% (e.g. in a FACS
- 35

based competition assay) of the maximum theoretical displacement (e.g. displacement by cold (e.g. unlabeled) antibody or fragment thereof that needs to be cross-blocked) by the potentially cross-blocking antibody or fragment thereof that is present in a given amount. Preferably, cross-competing antibodies or portions thereof have a recorded displacement
5 that is between 10% and 100%, or between 50% and 100%.

The anti-CSF1R antibody molecule or antigen-binding portion as defined herein may be thermally stable. In some cases, an antibody molecule or antigen-binding portion may have substantially the same thermal stability as murine anti-CSF1R antibody 0301 or
10 hu0301 (humanized). In some cases, an antibody molecule or antigen-binding portion may be more thermally stable than murine anti-CSF1R antibody 0301 or hu0301 (humanized). In some cases, an antibody molecule or antigen-binding portion may be less thermally stable than murine anti-CSF1R antibody 0301 or hu0301 (humanized). In some examples, an antibody molecule or antigen-binding portion may have a T_m from about 76°C to about
15 78°C and may be in an IgG4(S228P) format. In some aspects, an antibody molecule or antigen-binding portion may have a T_m from about 76.6°C to about 77.7°C and may be in an IgG4(S228P) format. The melting temperature of an antibody molecule or antigen-binding portion thereof may be analysed by a differential scanning calorimetry (DSC) assay.

20 The anti-CSF1R antibody molecule or antigen-binding portion as defined herein may be resistant to oxidation (e.g., resistant to oxidation of exposed amino acid residues). In some cases, an antibody molecule or antigen-binding portion may undergo reduced oxidation of exposed amino acid residues compared to murine anti-CSF1R antibody 0301 or hu0301
25 (humanized), or an anti-CSF1R antibody comprising the variable domain sequences of antibody hu0301 (humanized). Oxidation resistance of an antibody molecule or antigen-binding portion thereof may be analysed by adding an oxidative reagent (e.g., H_2O_2) to the antibody molecule or antigen-binding portion and analysing changes induced by oxidation by Reverse Phase (RP) Chromatography methods.

30 The anti-CSF1R antibody molecule or antigen-binding portion as defined herein may exhibit lower heterogeneity of hydrophobic species. In some cases, an antibody molecule or antigen-binding portion may exhibit reduced heterogeneity of hydrophobic species compared to murine anti-CSF1R antibody 0301 or hu0301 (humanized), or an anti-CSF1R
35 antibody comprising the variable domain sequences of antibody hu0301 (humanized). The

hydrophobic species heterogeneity of an antibody molecule or antigen-binding portion thereof may be analysed by Hydrophobic Interaction Chromatography (HIC) methods.

5 In some embodiments, an anti-CSFR1 antibody molecule or antigen-binding portion provided herein does not bind or minimally binds to insulin and/or dsDNA. In some embodiments, the insulin is human insulin. In some embodiments, an anti-CSFR1 antibody molecule or antigen-binding portion provided herein exhibits no binding to insulin and/or dsDNA above background binding. In some embodiments, an anti-CSFR1 antibody molecule or antigen-binding portion exhibits reduced binding to insulin and/or dsDNA compared to the binding
10 exhibited by Bococizumab, Briakinumab, Ustekinumab or Bevacizumab, or any combination of these antibodies. In some embodiments, binding to insulin or dsDNA may be determined by ELISA.

15 The antibody molecule or antigen-binding portion as defined herein may comprise one or more substitutions, deletions and/or insertions which remove a post-translational modification (PTM) site, for example a glycosylation site (N-linked or O-linked), a deamination site, a phosphorylation site or an isomerisation/fragmentation site.

20 More than 350 types of PTM are known. Key forms of PTM include phosphorylation, glycosylation (*N*- and *O*-linked), sumoylation, palmitoylation, acetylation, sulfation, myristoylation, prenylation and methylation (of K and R residues). Statistical methods to identify putative amino acid sites responsible for specific PTMs are well known in the art (see Zhou *et al.*, 2016, Nature Protocols 1: 1318-1321). Removal of such a site for example by substitution, deletion and/or insertion and then optionally testing (experimentally and/or
25 theoretically) for (a) binding activity and/or (b) loss of the PTM is contemplated.

For example, the 0301 murine LCDR1 (as defined herein, i.e. the amino acid sequence KASQSVDDYDGDNYMN; SEQ ID NO:22) has been identified to have a putative isomerization site at residues 9 and 10 (DG). Removal of this site at equivalent positions in an LCDR1 of
30 the invention, for example by conservative or non-conservative substitution of the D (such as to E, Q, H), is envisaged (as for example in clone 30E06 and other clones in Tables 3 and 4).

35 The antibody molecule or antigen-binding portion thereof may be human, humanized or chimeric.

The antibody molecule or antigen-binding portion thereof may comprise one or more human variable domain framework scaffolds into which the CDRs have been inserted. For example, the VH region, the VL region, or both the VH and the VL region may comprise one or more human framework region amino acid sequences.

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The antibody molecule or antigen-binding portion thereof may comprise an IGHV1-69 human germline scaffold into which the corresponding HCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VH region that comprises an IGHV1-69 human germline scaffold amino acid sequence into which a set of corresponding HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted. In some embodiments, the antibody molecule or antigen-binding portion of the invention comprises the IGHV1-69*01 allele germline framework regions in the VH domain.

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The antibody molecule or antigen-binding portion thereof may comprise an IGKV3-11 human germline scaffold into which the corresponding LCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VL region that comprises an IGKV3-11 human germline scaffold amino acid sequence into which a set of corresponding LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted.

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The antibody molecule or antigen-binding portion thereof may comprise an IGHV1-69 human germline scaffold into which the corresponding HCDR sequences have been inserted and an IGKV3-11 human germline scaffold into which the corresponding LCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VH region that comprises an IGHV1-69 human germline scaffold amino acid sequence into which a set of corresponding HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted and a VL region that comprises an IGKV3-11 human germline scaffold amino acid sequence into which a set of corresponding LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted. The HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 amino acid sequences may be the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 amino acid sequences of any one of the clones in Table 4 (with all six CDR sequences being from the same clone).

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In some aspects, the antibody molecule or antigen-binding portion thereof may comprise an immunoglobulin constant region. In some embodiments, the immunoglobulin constant region is IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2. In additional embodiments, the immunoglobulin constant region is IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2. The antibody

molecule or antigen-binding portion thereof may comprise an immunologically inert constant region. In some aspects, an anti-CSFR1 antibody or antigen-binding portion thereof may comprise an immunoglobulin constant region comprising a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A, G237A and P331S. In some aspects, an anti-CSFR1 antibody may comprise an immunoglobulin constant region comprising any one of the amino acid sequences in Table 17. The Fc region sequences in Table 17 begin at the CH1 domain. In some aspects, an anti-CSFR1 antibody may comprise an immunoglobulin constant region comprising an amino acid sequence of an Fc region of human IgG4, human IgG4(S228P), human IgG1, human IgG1-3M or human IgG1-4M. For example, the human IgG4(S228P) Fc region comprises the following substitution compared to the wild-type human IgG4 Fc region: S228P. For example, the human IgG1-3M Fc region comprises the following substitutions compared to the wild-type human IgG1 Fc region: L234A, L235A and G237A, while the human IgG1-4M Fc region comprises the following substitutions compared to the wild-type human IgG1 Fc region: L234A, L235A, G237A and P331S. In some aspects, a position of an amino acid residue in a constant region of an immunoglobulin molecule is numbered according to EU nomenclature (Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94). In some aspects, an immunoglobulin constant region may comprise an RDEL (SEQ ID NO:72) motif or an REEM (SEQ ID NO:73) motif (underlined in Table 17). The REEM (SEQ ID NO:73) allotype is found in a smaller human population than the RDEL (SEQ ID NO:72) allotype. In some aspects, an anti-CSFR1 antibody may comprise an immunoglobulin constant region comprising any one of SEQ ID NOS:359-364. In some aspects, an anti-CSFR1 antibody may comprise the six CDR amino acid sequences of any one of the clones in Table 4 and any one of the Fc region amino acid sequences in Table 17. In some aspects, an anti-CSFR1 antibody may comprise an immunoglobulin heavy chain constant region comprising any one of the Fc region amino acid sequences in Table 17 and an immunoglobulin light chain constant region that is a kappa light chain constant region or a lambda light chain constant region.

In some aspects, disclosed herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region and a heavy chain constant region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32) and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38)

and LCDR3 of QLSNEDLLT (SEQ ID NO: 39); and the heavy chain constant region comprises any one of SEQ ID NOS:359-364;

(b) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60) and LCDR3 of QLSNEDLLT (SEQ ID NO: 39); and the heavy chain constant region comprises any one of SEQ ID NOS:359-364;

(c) the VH region amino acid sequence comprises HCDR1 of GYTFSSAYMI (SEQ ID NO:57), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32) and HCDR3 of EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33); the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO:37), LCDR2 of AASDRAT (SEQ ID NO:60) and LCDR3 of QLSNEDLLT (SEQ ID NO:39); and the heavy chain constant region comprises any one of SEQ ID NOS:359-364; or

(d) the VH region amino acid sequence comprises HCDR1 of GYTFTSYMI (SEQ ID NO:41), HCDR2 of MGDINPYNGGTTYAQKFQG (SEQ ID NO:42) and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO:33); the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLA (SEQ ID NO:40), LCDR2 of AASNLAT (SEQ ID NO:43) and LCDR3 of QLSNEDLLT (SEQ ID NO:39); and the heavy chain constant region comprises any one of SEQ ID NOS:359-364.

In some aspects, disclosed herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region, a light chain variable (VL) region and a heavy chain constant region, wherein

(a) the VH region amino acid sequence comprises or consists of SEQ ID NO:352; the VL region amino acid sequence comprises or consists of SEQ ID NO:351; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A;

(b) the VH region amino acid sequence comprises or consists of SEQ ID NO:354; the VL region amino acid sequence comprises or consists of SEQ ID NO:353; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A;

(c) the VH region amino acid sequence comprises or consists of SEQ ID NO:356; the VL region amino acid sequence comprises or consists of SEQ ID NO:355; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A; or

(d) the VH region amino acid sequence comprises or consists of SEQ ID NO:358; the VL region amino acid sequence comprises or consists of SEQ ID NO:357; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A.

In some aspects, disclosed herein is an anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region and a heavy chain constant region, wherein

(a) the VH region amino acid sequence comprises or consists of SEQ ID NO:352; the VL region amino acid sequence comprises or consists of SEQ ID NO:351; and the heavy chain constant region comprises any one of SEQ ID NOS:359-364;

(b) the VH region amino acid sequence comprises or consists of SEQ ID NO:354; the VL region amino acid sequence comprises or consists of SEQ ID NO:353; and the heavy chain constant region comprises any one of SEQ ID NOS:359-364;

(c) the VH region amino acid sequence comprises or consists of SEQ ID NO:356; the VL region amino acid sequence comprises or consists of SEQ ID NO:355; and the heavy chain constant region comprises any one of SEQ ID NOS:359-364; or

(d) the VH region amino acid sequence comprises or consists of SEQ ID NO:358; the VL region amino acid sequence comprises or consists of SEQ ID NO:357; and the heavy chain constant region comprises any one of SEQ ID NOS:359-364.

In some aspects, an anti-CSFR1 antibody may be immune effector null. In some aspects, an anti-CSFR1 antibody or an antigen-binding portion thereof does not induce immune effector function and, optionally, suppresses immune effector function. In some aspects, an anti-CSFR1 antibody may lack measurable binding to human Fc γ RI, Fc γ RIIa, Fc γ RIIIa and Fc γ RIIIb receptors but maintain binding to human Fc γ RIIIb receptor and optionally maintain binding to human FcRn receptor. Fc γ RI, Fc γ RIIa, Fc γ RIIIa and Fc γ RIIIb are examples of activating receptors. Fc γ RIIIb is an example of an inhibitory receptor. FcRn is an example

of a recycling receptor. In some aspects, binding affinity of an anti-CSFR1 antibody or an antigen-binding portion thereof for human Fc receptors may be measured by BIACORE® analysis. In some aspects, Homogeneous Time Resolved Fluorescence (HTRF) can be used to study binding of an anti-CSFR1 antibody to human Fc receptors. In one example of
5 HTRF, human IgG1 (wild type) is labelled, as is the full suite of Fc gamma receptors and then antibodies with engineered Fc fragments are used in titration competition. In some aspects, CSFR1-positive cells may be mixed with human white blood cells and anti-CSFR1 antibodies, and cell killing by CDC, ADCC and/or ADCP may be measured. In some aspects, an anti-CSFR1 antibody comprising an amino acid sequence of an Fc region of human IgG1-
10 3M (see Table 17) is effector null. In some aspects, an anti-CSFR1 antibody comprising an amino acid sequence of an Fc region of human IgG1-3M (see Table 17) is not effector null.

The antibody molecule or antigen-binding portion thereof may be a Fab fragment, a F(ab)₂ fragment, an Fv fragment, a tetrameric antibody, a tetravalent antibody, a multispecific
15 antibody (for example, a bivalent antibody), a domain-specific antibody, a single domain antibody, a monoclonal antibody or a fusion protein. In one embodiment, an antibody may be a bispecific antibody that binds specifically to a first antigen and a second antigen, wherein the first antigen is CSF1R and the second antigen is not CSF1R. Antibody molecules and methods for their construction and use are described, in for example Holliger & Hudson
20 (2005, Nature Biotechnol. 23(9): 1126-1136).

In another aspect of the invention, there is provided an immunoconjugate comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein linked
25 a therapeutic agent.

Examples of suitable therapeutic agents include cytotoxins, radioisotopes, chemotherapeutic agents, immunomodulatory agents, anti-angiogenic agents, antiproliferative agents, pro-apoptotic agents, and cytostatic and cytolytic enzymes (for example RNAses). Further
30 therapeutic agents include a therapeutic nucleic acid, such as a gene encoding an immunomodulatory agent, an anti-angiogenic agent, an anti-proliferative agent, or a pro-apoptotic agent. These drug descriptors are not mutually exclusive, and thus a therapeutic agent may be described using one or more of the above terms.

Examples of suitable therapeutic agents for use in immunoconjugates include the taxanes,
35 maytansines, CC-1065 and the duocarmycins, the calicheamicins and other enediynes, and the auristatins. Other examples include the anti-folates, vinca alkaloids, and the

anthracyclines. Plant toxins, other bioactive proteins, enzymes (i.e., ADEPT), radioisotopes, photosensitizers may also be used in immunoconjugates. In addition, conjugates can be made using secondary carriers as the cytotoxic agent, such as liposomes or polymers. Suitable cytotoxins include an agent that inhibits or prevents the function of cells and/or results in destruction of cells. Representative cytotoxins include antibiotics, inhibitors of tubulin polymerization, alkylating agents that bind to and disrupt DNA, and agents that disrupt protein synthesis or the function of essential cellular proteins such as protein kinases, phosphatases, topoisomerases, enzymes, and cyclins.

Representative cytotoxins include, but are not limited to, doxorubicin, daunorubicin, idarubicin, aclarubicin, zorubicin, mitoxantrone, epirubicin, carubicin, nogalamycin, menogaril, pitarubicin, valrubicin, cytarabine, gemcitabine, trifluridine, ancitabine, enocitabine, azacitidine, doxifludine, pentostatin, broxuhdine, capecitabine, cladhbine, decitabine, floxuhdine, fludarabine, gougerotin, puromycin, tegafur, tiazofuhn, adhamycin, cisplatin, carboplatin, cyclophosphamide, dacarbazine, vinblastine, vincristine, mitoxantrone, bleomycin, mechlorethamine, prednisone, procarbazine, methotrexate, flurouracils, etoposide, taxol, taxol analogs, platins such as cis-platin and carbo-platin, mitomycin, thiotepa, taxanes, vincristine, daunorubicin, epirubicin, actinomycin, authramycin, azaserines, bleomycins, tamoxifen, idarubicin, dolastatins/auristatins, hemiasterlins, esperamicins and maytansinoids.

Suitable immunomodulatory agents include anti-hormones that block hormone action on tumors and immunosuppressive agents that suppress cytokine production, down-regulate self-antigen expression, or mask MHC antigens.

Also provided is a nucleic acid molecule encoding the antibody molecule or antigen-binding portion thereof of the invention as defined herein. A nucleic acid molecule may encode (a) the VH region amino acid sequence; (b) the VL region amino acid sequence; or (c) both the VH and the VL region amino acid sequences of an anti-CSF1R antibody or an antigen-binding portion thereof described herein. In some aspects, the nucleic acid molecule as defined herein may be isolated.

Further provided is a vector comprising the nucleic acid molecule of the invention as defined herein. The vector may be an expression vector.

Also provided is a host cell comprising the nucleic acid molecule or the vector of the invention as defined herein. The host cell may be a recombinant host cell.

5 In a further aspect there is provided a method of producing an anti-CSF1R antibody and/or an antigen-binding portion thereof, comprising culturing the host cell of the invention under conditions that result in expression and/or production of the antibody and/or the antigen-binding portion thereof, and isolating the antibody and/or the antigen-binding portion thereof from the host cell or culture.

10 In another aspect of the invention there is provided a pharmaceutical composition comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein.

15 Further provided is a method for enhancing an immune response in a subject, comprising administering to the subject an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention
20 as defined herein.

In a further aspect there is provided a method for treating or preventing cancer in a subject, comprising administering to the subject an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate
25 of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

The cancer may for example be selected from the group consisting of: pancreatic cancer,
30 melanoma, breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer,
35 thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, and cancer of hematological tissues. In one embodiment, the cancer is acute myeloid leukaemia.

The invention also provides an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein, for use in the treatment of cancer.

The invention further provides a method for depleting immunosuppressive CSF1R+ cells in the tumour microenvironment that inhibit the anti-tumour activity of T cells in a subject in need thereof, comprising administering to the subject an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

In another aspect the invention provides the antibody molecule, or antigen-binding portion thereof, or the immunoconjugate, or the nucleic acid molecule, or the vector for use, or the method of treatment of the invention as defined herein, for separate, sequential or simultaneous use in a combination combined with a second therapeutic agent, for example an anti-cancer agent. In some embodiments, a second therapeutic agent is a checkpoint inhibitor molecule. For example, a checkpoint inhibitor molecule may be an anti-PD1 antibody.

In a further aspect there is provided the use of an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or an immunoconjugate of the invention as defined herein, or a nucleic acid molecule of the invention as defined herein, or a vector of the invention as defined herein, or a pharmaceutical composition of the invention as defined herein, in the manufacture of a medicament for the treatment of cancer.

The invention also provides a method for treating or preventing an infectious or immune disease in a subject, comprising administering to the subject an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein. In one embodiment, the immune disease is pigmented villonodular synovitis. In some embodiments, the infectious disease is viral, bacterial, fungal or parasitic.

In one embodiment, the invention provides an anti-CSF1R antibody or an antigen-binding portion thereof comprising the amino acid sequences disclosed herein for use in therapy.

- 5 The pharmaceutical composition of the invention may comprise a pharmaceutically acceptable excipient, carrier, or diluent. A pharmaceutically acceptable excipient may be a compound or a combination of compounds entering into a pharmaceutical composition which does not provoke secondary reactions and which allows, for example, facilitation of the administration of the anti-CSF1R antibody molecule, an increase in its lifespan and/or in its efficacy in the body or an increase in its solubility in solution. These pharmaceutically acceptable vehicles are well known and will be adapted by the person skilled in the art as a function of the mode of administration of the anti-CSF1R antibody molecule.

- 15 In some embodiments, the anti-CSF1R antibody molecule may be provided in a lyophilised form for reconstitution prior to administration. For example, lyophilised antibody molecules may be re-constituted in sterile water and mixed with saline prior to administration to an individual.

- 20 The anti-CSF1R antibody molecules will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the antibody molecule. Thus pharmaceutical compositions may comprise, in addition to the anti-CSF1R antibody molecule, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the anti-CSF1R antibody molecule. The precise nature of the carrier or other material will depend on the route of administration, which may be by bolus, infusion, injection or any other suitable route, as discussed below.

- 30 For parenteral, for example sub-cutaneous or intra-venous administration, e.g. by injection, the pharmaceutical composition comprising the anti-CSF1R antibody molecule may be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles, such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be employed as required including buffers such as phosphate, citrate and other organic acids; antioxidants, such as 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 polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone; amino acids, such as glycine, glutamine, asparagines, 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 TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

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A pharmaceutical composition comprising an anti-CSF1R antibody molecule may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

15 An anti-CSF1R antibody molecule as described herein may be used in a method of treatment of the human or animal body, including prophylactic or preventative treatment (e.g. treatment before the onset of a condition in an individual to reduce the risk of the condition occurring in the individual; delay its onset; or reduce its severity after onset). The method of treatment may comprise administering the anti-CSF1R antibody molecule to an individual in need thereof.

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Administration is normally in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is 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 composition, the method of administration, the scheduling of administration and other factors known to medical practitioners. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors and may depend on the severity of the symptoms and/or progression of a disease being treated. Appropriate doses of antibody molecules are well known in the art (Ledermann J.A. *et al.*, 1991, *Int. J. Cancer* 47: 659-664; Bagshawe K.D. *et al.*, 1991, *Antibody, Immunoconjugates and Radiopharmaceuticals* 4: 915-922). Specific dosages may be indicated herein or in the Physician's Desk Reference (2003) as appropriate for the type of medicament being administered may be used. A therapeutically effective amount or suitable dose of an antibody molecule may be determined by comparing its *in vitro* activity and *in vivo* activity in

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an animal model. Methods for extrapolation of effective dosages in mice and other test animals to humans are known. The precise dose will depend upon a number of factors, including whether the antibody is for prevention or for treatment, the size and location of the area to be treated, the precise nature of the antibody (e.g. whole antibody, fragment) and the nature of any detectable label or other molecule attached to the antibody.

A typical antibody dose will be in the range 100 µg to 1 g for systemic applications, and 1 µg to 1 mg for topical applications. An initial higher loading dose, followed by one or more lower doses, may be administered. Typically, the antibody will be a whole antibody, e.g. the IgG1 or IgG4 isotype. This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children and infants, and also adjusted for other antibody formats in proportion to molecular weight. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician. The treatment schedule for an individual may be dependent on the pharmacokinetic and pharmacodynamic properties of the antibody composition, the route of administration and the nature of the condition being treated.

Treatment may be periodic, and the period between administrations may be about two weeks or more, e.g. about three weeks or more, about four weeks or more, about once a month or more, about five weeks or more, or about six weeks or more. For example, treatment may be every two to four weeks or every four to eight weeks. Treatment may be given before, and/or after surgery, and/or may be administered or applied directly at the anatomical site of surgical treatment or invasive procedure. Suitable formulations and routes of administration are described above.

In some embodiments, anti-CSF1R antibody molecules as described herein may be administered as sub-cutaneous injections. Sub-cutaneous injections may be administered using an auto-injector, for example for long term prophylaxis/treatment.

In some preferred embodiments, the therapeutic effect of the anti-CSF1R antibody molecule may persist for several half-lives, depending on the dose. For example, the therapeutic effect of a single dose of the anti-CSF1R antibody molecule may persist in an individual for 1 month or more, 2 months or more, 3 months or more, 4 months or more, 5 months or more, or 6 months or more.

The invention also provides a method of producing an antibody molecule which specifically binds to human CSF1R and optionally also to cynomolgus and rhesus monkey CSF1R, or an antigen-binding portion thereof, comprising the steps of:

- (1) grafting anti-CSF1R CDRs from a non-human source into a human v-domain framework to produce a humanized anti-CSF1R antibody molecule or antigen-binding portion thereof;
- (2) generating a library of clones of the humanized anti-CSF1R antibody molecule or antigen-binding portion thereof comprising one or more mutations in the CDRs;
- (3) screening the library for binding to human CSF1R and optionally also to cynomolgus and rhesus monkey CSF1R;
- (4) selecting clones from the screening step (3) having binding specificity to human CSF1R and optionally also to cynomolgus and rhesus monkey CSF1R; and
- (5) producing an antibody molecule which specifically binds to human CSF1R and optionally also to cynomolgus and rhesus monkey CSF1R, or an antigen-binding portion thereof from clones selected from step (4).

15

The method may comprise a further step of producing additional clones based on the clones selected in step (4), for example based on further exploratory mutagenesis at specific positions in the CDRs of the clones selected in step (4), to enhance humanization and/or minimise human T cell epitope content and/or improve manufacturing properties in the antibody molecule or antigen-binding portion thereof produced in step (5).

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Refinements applicable to the above method are as described in Example 1 below.

As used herein, the term "CSF1R" refers to Colony Stimulating Factor 1 Receptor and variants thereof that retain at least part of the biological activity of CSF1R. As used herein, CSF1R includes all species of native sequence CSF1R, including human, rat, mouse and chicken. The term "CSF1R" is used to include variants, isoforms and species homologs of human CSF1R. Antibodies of the invention may cross-react with CSF1R from species other than human, in particular CSF1R from cynomolgus monkey (*Macaca fascicularis*) and rhesus monkey (*Macaca mulatta*). In certain embodiments, the antibodies may be completely specific for human CSF1R and may not exhibit non-human cross-reactivity.

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As used herein, an "antagonist" as used in the context of the antibody of the invention or an "anti-CSF1R antagonist antibody" (interchangeably termed "anti-CSF1R antibody") refers to an antibody which is able to bind to CSF1R and inhibit CSF1R biological activity and/or downstream pathway(s) mediated by CSF1R signalling. An anti-CSF1R antagonist antibody

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encompasses antibodies that can block, antagonize, suppress or reduce (including significantly) CSF1R biological activity, including downstream pathways mediated by CSF1R signalling, such as receptor binding and/or elicitation of a cellular response to CSF1R. For the purposes of the present invention, it will be explicitly understood that the term “anti-CSF1R antagonist antibody” encompass all the terms, titles, and functional states and characteristics whereby CSF1R itself, and CSF1R biological activity (including but not limited to its ability to modulate the activity of mononuclear cells), or the consequences of the activity or biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree.

CSF1R “specifically binds” “specifically interacts”, “preferentially binds”, “binds” or “interacts” with CSF1R if it binds with greater affinity, avidity, more readily and/or with greater duration than it binds to other receptors.

An “antibody molecule” is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term “antibody molecule” encompasses not only intact polyclonal or monoclonal antibodies, but also any antigen binding fragment (for example, an “antigen-binding portion”) or single chain thereof, fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site including, for example without limitation, scFv, single domain antibodies (for example, shark and camelid antibodies), maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv.

An “antibody molecule” encompasses an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), for example IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

- The term “antigen binding portion” of an antibody molecule, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to CSF1R. Antigen binding functions of an antibody molecule can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term “antigen binding portion” of an antibody molecule include Fab; Fab'; F(ab')₂; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment, and an isolated complementarity determining region (CDR).
- 10 The term “Fc region” is used to define a C-terminal region of an immunoglobulin heavy chain. The “Fc region” may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3. As is known in the art, an Fc region can be present in dimer or monomeric form.
- 20 A “variable region” of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four framework regions (FRs) connected by three complementarity determining regions (CDRs) also known as hypervariable regions, contribute to the formation of the antigen binding site of antibodies. When choosing FR to flank CDRs, for example when humanizing or optimizing an antibody, FRs from antibodies which contain CDR sequences in the same canonical class are preferred.
- 30 The CDR definitions used in the present application combine the domains used in the many disparate, often conflicting schemes that have been created in the field, which are based on the combination of immunoglobulin repertoire analyses and structural analyses of antibodies in isolation and in their co-crystals with antigens (see review by Swindells *et al.*, 2016, abYsis: Integrated Antibody Sequence and Structure-Management, Analysis, and Prediction. J Mol Biol. [PMID: 27561707; Epub 22 August 2016]). The CDR definition used herein (a “Unified” definition) incorporates the lessons of all such prior insights and includes all appropriate loop positions required to sample the full residue landscape that potentially mediates target-binding complementarity.

Table 1 shows the amino acid sequences of the 0301 murine anti-CSF1R antibody CDRs as defined herein (a “Unified” scheme), in comparison to well-known alternative systems for defining the same CDRs.

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As used herein the term “conservative substitution” refers to replacement of an amino acid with another amino acid which does not significantly deleteriously change the functional activity. A preferred example of a “conservative substitution” is the replacement of one amino acid with another amino acid which has a value ≥ 0 in the following BLOSUM 62 substitution

10 matrix (see Henikoff & Henikoff, 1992, PNAS 89: 10915-10919):

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
15 N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	5	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
20 G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	8	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5	-1	-3	-1	0	-1	-3	-2	-2
25 M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5	-2	-2	0
30 W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4.

The term “monoclonal antibody” (Mab) refers to an antibody, or antigen-binding portion thereof, that is derived from a single copy or clone, including for example any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Preferably, a monoclonal antibody of the invention exists in a homogeneous or substantially homogeneous population.

40 A “humanized” antibody molecule refers to a form of non-human (for example, murine) antibody molecules, or antigen-binding portion thereof, that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies may be human immunoglobulins (recipient

antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity.

- 5 “Human antibody or fully human antibody” refers to an antibody molecule, or antigen-binding portion thereof, derived from transgenic mice carrying human antibody genes or from human cells.

- 10 The term “chimeric antibody” is intended to refer to an antibody molecule, or antigen-binding portion thereof, in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody molecule in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

- 15 “Antibody-drug conjugate” and “immunoconjugate” refer to an antibody molecule, or antigen-binding portion thereof, including antibody derivatives that binds to CSF1R and is conjugated to cytotoxic, cytostatic and/or therapeutic agents.

- 20 Antibody molecules of the invention, or antigen-binding portion thereof, can be produced using techniques well known in the art, for example recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art.

- 25 The term “isolated molecule” (where the molecule is, for example, a polypeptide, a polynucleotide, or an antibody) is a molecule that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or expressed in a cellular system different from the cell from which
30 it naturally originates, will be “isolated” from its naturally associated components. A molecule also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to
35 visualize the polypeptide using techniques well known in the art. For certain purposes, higher

resolution may be provided by using HPLC or other means well known in the art for purification.

5 The term "epitope" refers to that portion of a molecule capable of being recognized by and bound by an antibody molecule, or antigen-binding portion thereof, at one or more of the antibody molecule's antigen-binding regions. Epitopes can consist of defined regions of primary secondary or tertiary protein structure and includes combinations of secondary structural units or structural domains of the target recognised by the antigen binding regions of the antibody, or antigen-binding portion thereof. Epitopes can likewise consist of a defined
10 chemically active surface grouping of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. The term "antigenic epitope" as used herein, is defined as a portion of a polypeptide to which an antibody molecule can specifically bind as determined by any method well known in the art, for example, by conventional immunoassays, antibody
15 competitive binding assays or by x-ray crystallography or related structural determination methods (for example NMR).

The term "binding affinity" or "KD" refers to the dissociation rate of a particular antigen-antibody interaction. The KD is the ratio of the rate of dissociation, also called the "off-rate
20 (k_{off})", to the association rate, or "on-rate (k_{on})". Thus, K_D equals $k_{\text{off}} / k_{\text{on}}$ and is expressed as a molar concentration (M). It follows that the smaller the K_D , the stronger the affinity of binding. Therefore, a K_D of 1 μM indicates weak binding affinity compared to a K_D of 1 nM. KD values for antibodies can be determined using methods well established in the art. One method for determining the KD of an antibody is by using surface plasmon resonance (SPR),
25 typically using a biosensor system such as a Biacore® system.

The term "potency" is a measurement of biological activity and may be designated as IC_{50} , or effective concentration of an antibody or antibody drug conjugate to the antigen CSF1R to inhibit 50% of activity measured in a CSF1R activity assay as described herein.

30 The phrase "effective amount" or "therapeutically effective amount" as used herein refers to an amount necessary (at dosages and for periods of time and for the means of administration) to achieve the desired therapeutic result. An effective amount is at least the minimal amount, but less than a toxic amount, of an active agent which is necessary to impart
35 therapeutic benefit to a subject.

The term “inhibit” or “neutralize” as used herein with respect to bioactivity of an antibody molecule of the invention means the ability of the antibody to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, eliminate, stop, reduce or reverse for example progression or severity of that which is being inhibited including, but not limited to, a biological activity or binding interaction of the antibody molecule to CSF1R.

A “host cell” includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

As used herein, “vector” means a construct, which is capable of delivering, and, preferably, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

The term “treating”, as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, delaying the progression of, delaying the onset of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. The term “treatment”, as used herein, unless otherwise indicated, refers to the act of treating as defined above. The term “treating” also includes adjuvant and neoadjuvant treatment of a subject. For the avoidance of doubt, reference herein to “treatment” includes reference to curative, palliative and prophylactic treatment. For the avoidance of doubt, references herein to “treatment” also include references to curative, palliative and prophylactic treatment.

It is understood that wherever embodiments are described herein with the language “comprising,” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided.

Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire

group listed as a whole, but each member of the group individually and all possible subgroups of the main group, but also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

5

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present specification, including definitions, will control. Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Any example(s) following the term "e.g." or "for example" is not meant to be exhaustive or limiting.

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The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art.

20

Particular non-limiting embodiments of the present invention will now be described with reference to accompanying drawings.

EXAMPLE 1. Generation of optimized anti-CSF1R therapeutic antibodies

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Introduction

In this example, we successfully generate a panel of agonistic, optimized anti-CSF1R antibodies. These anti-CSF1R antibodies are well expressed, biophysically stable, highly soluble and of maximized identity to preferred human germlines.

30

Materials and methods

CSF1R library generation and selection

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The CSF1R Fab repertoire was assembled by mass oligo synthesis and PCR. The amplified Fab repertoire was then cloned via restriction-ligation into a phagemid vector, transformed into *E.coli* TG-1 cells, and the phage repertoire rescued essentially as previously described in detail (Finlay *et al.*, 2011, Methods Mol Biol 681: 383-401).

Phage selections were performed by coating streptavidin magnetic microbeads with biotinylated CSF1R target protein (either human or cyno), washing the beads thrice with PBS and resuspending in PBS pH7.4 plus 5% skim milk protein. These beads were coated at 100 nM target protein in round 1 of selection, followed by reduced antigen concentrations in three successive rounds. In each round, phage were eluted using trypsin before re-infection into TG1 cells.

Periplasmic extracts production (small-scale)

Production of soluble Fabs in individual *E. coli* clones was performed. *E. coli* TG1 cells in logarithmic growth phase were induced with isopropyl 1-thio- β -D-galactopyranoside. Periplasmic extracts containing soluble Fab were generated by a freeze/thaw cycle: Bacterial cell pellets were frozen at -20 °C for overnight and then thawed at room temperature and resuspended in PBS pH 7.4. The supernatants containing the soluble Fab were collected after shaking at room temperature and centrifugation.

IgG expression and purification

Mammalian codon-optimized synthetic genes encoding the heavy and light chain variable domains of the lead panel anti-CSF1R antibodies plus the mu0301 and hu0301 were cloned into mammalian expression vectors comprising human IgG4-S228P (human IgG4 containing the S228P mutation in the hinge region that stabilises the hinge) and human C κ domains, respectively. Co-transfection of heavy and light chain containing vector in mammalian expression system was performed, followed by protein A-based purification of the IgG, quantification and QC on denaturing and non-denaturing SDS-PAGE.

Direct binding ELISA for Fab and IgG

Binding and cross-reactivity of the lead panel to the recombinant proteins was initially assessed by binding ELISA. The human CSF1R human Fc tagged recombinant protein and the cynomolgus monkey CSF1R human Fc tagged recombinant protein were coated to the surface of MaxiSorp™ flat-bottom 96 well plate at 1 μ g/ml. The purified IgG samples were titrated in two fold serial dilutions starting from 500 nM to 0.008 nM and allowed to bind to the coated antigens. The Fabs were detected using mouse anti-c-myc antibody followed by donkey anti-mouse IgG conjugated to horseradish peroxidase. The IgGs were detected using the mouse anti-human IgG conjugated to horseradish peroxidase. Binding signals were visualized with 3,3',5,5'-Tetramethylbenzidine Substrate Solution (TMB) and the absorbance measured at 450 nm.

Alphascreen epitope competition assay for IgG antibodies

The AlphaScreen assay (Perkin Elmer) was performed in a 25 µl final volume in 384-well white microtiter plates (Greiner). The reaction buffer contained 1xPBS pH 7.3 (Oxoid, Cat. nr. BR0014G) and 0.05 % (v/v) Tween® 20 (Sigma, Cat. nr. P9416). Purified IgG samples were titrated in three fold serial dilutions starting at 500 nM final concentration and incubated with biotinylated human CSF1R for 20 minutes at room temperature. The mu0301 IgG at and the anti-human IgG Acceptor beads were added and the mix was incubated for 1 hour at room temperature, followed by addition of the Streptavidin Donor beads at 20 µg/ml (final concentration) and incubation for 30 minutes at room temperature. The emission of light was measured in the EnVision multilabel plate reader (Perkin Elmer) and analysed using the EnVision manager software. Values were reported as Counts Per Second (CPS) and corrected for crosstalk. The EC50 values were calculated using the MFI values in GraphPad Prism software (GraphPad Software, La Jolla, CA).

15

Biacore analyses of IgG affinity for monomeric human and cyno CSF1R in solution

Affinity (KD) of purified IgGs was determined via SPR with antigen in-solution on a Biacore 3000 (GE). A mouse anti-human antibody (CH1 specific) was immobilized on a CM5 Sensor Chip to a level of 2000 RU in acetate buffer at pH 4.5 using amine coupling following the Wizard instructions for two channels. One channel was used for background signal correction. The standard running buffer HBS-EP pH 7.4 was used. Regeneration was performed with a single injection of 10 µl of 10 mM Glycine at pH 1.5 at 20 µl/minute. IgG samples were injected for 2 minutes at 50 nM at 30 µl/min followed by and off-rate of 60 seconds. The monomeric ectodomain protein (human CSF1R or cynomolgus monkey CSF1R) was injected in two fold serial dilutions from 100 nM down to 3.1 nM, for 2 minutes at 30 µl/min followed by an off-rate of 300 seconds. The obtained sensorgrams were analysed using the Biacore 3000 evaluation (BIAevaluation) software. The KD was calculated by simultaneous fitting of the association and dissociation phases to a 1:1 Langmuir binding model.

30

Flow cytometry of IgGs

Purified IgGs were tested in FACs for binding to human and cyno CSF1R expressed on HEK-293 cells. The IgG samples were titrated in three-fold serial dilutions starting at 500 nM to 0.98 nM. Binding of IgGs was detected with a mouse anti-human IgG conjugated to FITC. Results were analyzed by examining the Mean Fluorescence Intensity (MFI) of 10000 cells

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per sample in the BL-1 channel detector of a flow cytometer (Attune™ NxT Acoustic Focusing Cytometer, Invitrogen/ ThermoFisher Scientific).

Antibody v-domain T cell epitope content: *in silico* analyses

5 *In silico* technologies (Abzena, Ltd.), which are based on identifying the location of T cell epitopes in therapeutic antibodies and proteins, were used for assessing potential immunogenicity in antibody v-domains. iTope™ was used to analyse the VL and VH sequences of key leads for peptides with promiscuous high affinity binding to human MHC class II. Promiscuous high affinity MHC class II binding peptides are thought to correlate with
10 the presence of T cell epitopes that are high risk indicators for clinical immunogenicity of drug proteins. The iTope™ software predicts favourable interactions between amino acid side chains of a peptide and specific binding pockets (in particular pocket positions; p1, p4, p6, p7 and p9) within the open-ended binding grooves of 34 human MHC class II alleles. These alleles represent the most common HLA-DR alleles found world-wide with no
15 weighting attributed to those found most prevalently in any particular ethnic population. Twenty of the alleles contain the 'open' p1 configuration and 14 contain the 'closed' configuration where glycine at position 83 is replaced by a valine. The location of key binding residues is achieved by the *in silico* generation of 9mer peptides that overlap by eight amino acids spanning the test protein sequence. This process successfully discriminates with high
20 accuracy between peptides that either bind or do not bind MHC class II molecules.

In addition, the sequences were analysed using TCED™ (T Cell Epitope Database™) search for matches to T cell epitopes previously identified by *in vitro* human T cell epitope mapping analyses of other protein sequences. The TCED™ is used to search any test sequence
25 against a large (>10,000 peptides) database of peptides derived from unrelated protein and antibody sequences.

Primary human monocyte proliferation assay

Primary human CD14+ monocytes were freshly isolated from the peripheral blood of 3
30 healthy human donors. Monocyte samples were treated with 2400 IU/ml M-CSF in the presence or absence of titrated anti-CSF1R IgG4-S228P clones. After 5 days, the cell survival/proliferation was quantified using CCK-8 reagent. Plates were read at intervals from 1 to 3 hours after addition of CCK-8 and the time point at which vehicle treated wells had an OD450 nm of 2 to 2.5 was used for analysis.

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Differential scanning calorimetry (DSC) Analysis

The T_m of test articles was analysed using a MicroCal PEAQ-DSC (Malvern Instruments, Malvern, UK) running version 1.22 software. The samples were heated at a rate of 200 °C/hour over a range of 20-110°C. Thermal data was normalised based on protein concentration. The T_m of the protein was determined from the heating scan data.

Forced Oxidation Analyses

For forced oxidation analysis of digested IgGs: test articles were treated with 0.5 % H₂O₂ at room temperature for 2 hours. Native and oxidised IgG4(S228P) samples were digested with trypsin using the SMART Digest™ kit (ThermoFisher Scientific, Hemel Hempstead, UK) by following the manufacturer's protocol. The resulting tryptic peptides were immediately analysed by Reverse Phase chromatography. Chromatographic separation was performed using an Acquity UPLC CSH C18 Column, 130 Å, 1.7 µm, 2.1 mm × 150 mm (Waters, Elstree, UK) connected to a Dionex Ultimate 3000RS HPLC system (ThermoFisher Scientific, Hemel Hempstead, UK). The method consisted of a linear gradient from 95% buffer A (0.1% FA in H₂O) to 15% buffer B (0.085% FA in 75% acetonitrile) over 4 minutes, followed by a linear gradient from 15% buffer B to 60% buffer B over 22 minutes. The flow rate was 0.2 mL/minute and the temperature was maintained at 40 °C throughout the analysis. Detection was carried out by UV absorption at 280 nm.

Hydrophobic Interaction Chromatography (HIC) analyses

Chromatographic separation was performed using a TSKgel Butyl-NPR 4.6 mm × 35 mm HIC column (TOSOH Bioscience Ltd., Reading, UK) connected to a Dionex Ultimate 3000RS HPLC system (ThermoFisher Scientific, Hemel Hempstead, UK). The method consisted of a linear gradient from 60% Buffer A (100 mM sodium phosphate pH 7.0, 2 M ammonium sulphate) to 90% Buffer B (100 mM sodium phosphate pH 7.0) over 9 minutes. The flow rate was 1.2 mL/minute. Detection was carried out by UV absorption at 280 nm.

Results and Discussion

CDR grafting onto preferred human germline v-genes

The CDRs of an antagonistic murine anti-CSF1R IgG '0301' (mu0301; see WO2011/140249A2 and Table 2) were initially introduced to human germline immunoglobulin v-domain framework sequence scaffolds using CDR grafting. To bias our engineering efforts towards final lead therapeutic IgG compounds with optimal drug-like properties, we chose to graft the CDRs of the parental antibody onto "preferred" germline scaffolds IGHV1-69 and IGKV3-11, which are known to have good solubility and drug

development qualities, and are used at high frequency in the expressed human antibody repertoire. The IGHV1-69 germline gene is known to have a significant number of allelic variants in the human population. Human immune repertoire sequencing studies have shown the allele IGHV1-69*01 to be the most commonly expressed and, as such, it was chosen as the specific allele on which to base the CDR grafts and library design.

Those scaffolds and grafted CDR definitions are outlined in Table 2. The heavy and light chain sequences for murine anti-CSF1R antibody are also shown in Table 2. While this process of CDR grafting is well known, it is still problematic to predict whether a given set of human v-domain sequences will act as suitable acceptor frameworks for non-human CDR grafting. The use of unsuitable frameworks can lead to the loss of target binding function, protein stability issues or even impaired expression of the final IgG. The IGHV1-69/IGKV3-11 germline graft was therefore taken forward as the template for CDR mutagenesis and selection of improved clones.

Library generation and screening

The CDR-grafted IGHV1-69/IGKV3-11 v-domain sequences were combined into a Fab phage display format and a mutagenesis library cassette was generated by oligo synthesis and assembly. Mutagenesis libraries were synthesised to not only sample human germline and murine residues in the CDRs, but also to sample mutations (e.g. D/E/Q) that could facilitate the potential removal of amino acids in the LCDR1 which are not found in the human germline IGKV3-11 (motif 'YDGDN' (SEQ ID NO: 74)), that contain the high risk putative isomerisation motif 'DG'. This additional mutagenesis meant that two separate Fab libraries were initially constructed that sampled mutational diversity in either the heavy or light chain v-domain sequences in combination with the cognate paired grafted v-domain. Both VH and VL libraries were separately ligated into a phage display vector and transformed into *E. coli* via electroporation to generate 1.06×10^9 and 8.73×10^8 independent clones, respectively. Library build quality was verified by sequencing 96 clones per library. This sequencing data showed that the positions encoding either the murine or human germline residue at each position of variance had been effectively sampled at a frequency of approximately 50%, or 33% in positions where 3 amino acids had been sampled. Libraries were rescued using helper phage M13 and selections performed on biotinylated human and cynomolgus monkey CSF1R-Fc proteins in multiple separate branches. After an initial round of selection, the two pools of mutated VH and VL domains were recombined into a secondary library and three further rounds of standard selection performed, plus two 'hammer-hug' rounds.

Post-selection screening (Fig. 1) and DNA sequencing revealed the presence of 598 human and cyno CSF1R-binding Fab clones that exhibited strong binding to human and cyno CSF1R in ELISA. Amongst these 598 clones, the framework sequences remained fully germline while mutations were observed in all CDRs (Table 3). Lead clones were ranked
5 based on level of CDR germ-lining versus ELISA signals for binding to both human and cyno CSF1R-Fc. The v-domains of the 10 top clones from this ranking were then sub-cloned into IgG expression vectors for further testing as below (Table 4).

While germ-lining mutations were observed in all CDRs for the lead clones derived directly
10 from library selections, it remained possible that sequence analyses might allow further clones to be designed to have maximal humanization. The 598 sequence-unique hits with binding signals against human and cyno protein were therefore used to analyse the retention frequency for murine amino acids in the CDRs of this functionally characterized population. Positional amino acid retention frequency was expressed as a percentage found in the V_L
15 and V_H domains (Fig. 2A&B, respectively). Murine residues with RF < 75% were regarded as positions that are possibly not essential to the target-binding paratope and are likely to be open to germ-lining, in a series of combinatorial designs (Table 4). In the V_L domain, only 10 of 22 murine CDR residues derived from the 0301 sequence were retained with frequencies >75% (Fig. 2A). In the V_H domain (excluding the CDR-H3), only 8 of 17 murine residues in
20 the CDR-H1 and H2 exhibited retention frequency above 75% (Fig. 2B). Importantly, the DG amino acid motif at positions 9 and 10 in the LCDR1 of hu0301 is a high risk for isomerization and resulting structural instability. The data in Figure 2A and the sequence diversity shown in Table 3 demonstrated clearly that this motif could likely be replaced with sequences of higher chemical stability, such as EG, QG.

25 Designs containing only those murine residues with RF > 75% were given the prefix "MH" (MH = Maximally Humanized). In total, 9 designer V_H and 3 designer V_L domains were generated (MH1-MH9, Table 4). The MH clones were generated by gene synthesis and (along with the 10 library-derived clones outlined above and positive controls mu0301 and
30 hu0301), cloned into human expression vectors for production in IgG4(S228P) format. All IgGs were readily expressed and purified from transient transfections of mammalian cells.

Lead IgG specificity and potency characteristics

The purified IgGs described above were then tested for binding to human and cyno CSF1R-
35 Fc in direct titration ELISA format. This analysis demonstrated that several library-derived clones had human and cyno CSF1R binding profiles overlapping with, or improved over,

mu0301 (Fig. 3A&B). Notable exceptions were clones MH1-3, MH7-9, 30C11 and 29E11, all of which exhibited poor binding to both orthologs of CSF1R, demonstrating that the CDR mutations found in these designer and library-derived clones are disruptive to binding when used in combination. As the ELISA EC₅₀ values for directly-binding IgGs are strongly
5 influenced by avidity, rather than true 1:1 binding affinity, we then proceeded to perform higher-sensitivity, solution-phase epitope competition and Biacore binding affinity determinations, as outlined below.

An Alphascreen assay was established to allow the testing of IgGs for epitope competition
10 with mu0301 binding to biotinylated monomeric human CSF1R. In this assay, the top-performing library-derived and designer IgGs were more effectively differentiated via IC₅₀ values (Table 5). While the majority of clones exhibited equivalent or improved competition for the 0301 epitope over hu0301 (Fig. 4), two further designer clones (MH4 and MH6) exhibited significantly reduced epitope competition (>2-fold lower than hu0301).

15 Biacore analyses of binding affinity were performed for all IgGs to solution-phase, monomeric human and cyno CSF1R proteins (Table 6). These analyses showed that library-derived clones which gave the highest IC₅₀ values the Alphascreen assay also showed highest affinity binding to human and cyno CSF1R. Importantly, library-derived clones 30E06, 29H09
20 and 29D10 all exhibited unexpectedly improved binding affinities for both human and cyno CSF1R in comparison to hu0301. Importantly, these improvements in affinity partially normalised the human/cyno affinities for these clones to < 3-fold (all K_D values < 0.42 nM), as opposed to hu0301, which exhibited an > 3-fold differential (human K_D – 0.21 nM, cyno K_D – 0.66 nM). Affinity differentials of less than 3-fold between human and cyno target
25 orthologs are highly beneficial in pre-clinical drug development analyses as they allow more accurate design and interpretation of e.g. monkey safety, PK and PD modelling experiments. In addition, comparison of the affinities of clones MH4, MH5 AND MH6, which shared an identical heavy chain sequence, highlighted the influence of selected light chain residues on maintaining target binding affinity, including: LCDR1 position 9 (D/E/Q), LCDR2 position 1
30 (D/A), and LCDR3 position 5 (E/Q). The use of the residue Q in the LCDR3 reduced the binding affinity for human CSF1R from 0.22 nM (clone MH5) to 0.88 nM (MH6), despite the retention frequency for the parental residue E being only 60% in the analysis shown in Fig. 2A. The use of the residues Q in the LCDR1, D in the LCDR2 and Q in the LCDR3 reduced the binding affinity for human CSF1R from 0.22 nM (clone MH5) to 1.9 nM (MH4). These
35 findings highlighted the difficulty in deciding ‘*a priori*’ which CDR residues may or may not be

converted to human germline identity, when attempting to maximise the humanization level of individual clones.

Flow cytometric analyses of lead IgG binding specificity at the cell membrane

5 Antibodies to CSF1R were analysed for concentration-dependent binding at the cell surface via flow cytometry. Initial analyses were performed on HEK-293 cells transiently-transfected with human or rhesus CSF1R. These analyses showed that lead library-derived and designer clones exhibit strong concentration-dependent binding to membrane-presented human CSF1R with binding curves overlapping with, or improved over, the
10 hu0301 IgG (Fig 5A). Analyses performed on rhesus CSF1R-transfected cells further confirmed that several key library-derived and designer leads also exhibited highly similar binding curves that overlapped with, or improved over, the hu0301 IgG (Fig 5B). No binding signals were observed for any clone on untransfected HEK-293 cells.

15 Antibody v-domain T cell epitope analyses

In silico technologies (Abzena, Ltd.), which are based on identifying the location of T cell epitopes in therapeutic antibodies and proteins, were used for assessing the immunogenicity of both the hu0301 and lead antibody v-domains. Analysis of the v-domain sequences was performed with overlapping 9mer peptides (with each overlapping the last
20 peptide by 8 residues) which were tested against each of the 34 MHC class II allotypes. Each 9mer was scored based on the potential 'fit' and interactions with the MHC class II molecules. The peptide scores calculated by the software lie between 0 and 1. Peptides that produced a high mean binding score (>0.55 in the iTope™ scoring function) were highlighted and, if $>50\%$ of the MHC class II binding peptides (i.e. 17 out of 34 alleles) had
25 a high binding affinity (score >0.6), such peptides were defined as 'high affinity' MHC class II binding peptides which are considered a high risk for containing CD4+ T cell epitopes. Low affinity MHC class II binding peptides bind a high number of alleles ($>50\%$) with a binding score >0.55 (but without a majority >0.6). Further analysis of the sequences was performed using the TCED™. The sequences were used to interrogate the TCED™ by
30 BLAST search in order to identify any high sequence homology between peptides (T cell epitopes) from unrelated proteins/antibodies that stimulated T cell responses in previous *in vitro* T cell epitope mapping studies performed at Abzena Ltd.

Peptides were grouped into four classes: High Affinity Foreign ('HAF' – high
35 immunogenicity risk), Low Affinity Foreign ('LAF' – lower immunogenicity risk), TCED+ (previously identified epitope in TCED™ database), and Germline Epitope ('GE' - human

germline peptide sequence with high MHC Class II binding affinity). Germline Epitope 9mer peptides are unlikely to have immunogenic potential due to T cell tolerance, as validated by previous studies with a wide range of germline peptides. Importantly, such germline v-domain epitopes (aided further by similar sequences in the human antibody constant regions) also compete for MHC Class II occupancy at the membrane of antigen presenting cells, reducing the risk of foreign peptide presentation being sufficient to achieve the 'activation threshold' required for T cell stimulation. High GE content is therefore a beneficial quality in clinical development of an antibody therapeutic.

As shown in Table 7, key lead v-domains exhibited significant beneficial changes in peptide epitope content in comparison to hu0301. As the v-domain engineering process undertaken here had successfully selected for antibodies that maintained anti-CSF1R potency despite germlining many of the murine CDR residues included in the v-domains of hu0301 (Table 2), multiple epitopes found in the heavy and light chain v-domains of hu0301 could be ablated in library-derived and designer leads (Table 7). GE epitope content was also found to be increased (from 8 to ≥ 9 in all leads), and TCED+ epitope content was reduced or eliminated in all leads other than clone 30E06 (Table 7). Importantly, multiple foreign epitopes were eliminated by germlining mutations found in the CDRs of lead clones. For example, a TCED+ and LAF peptide 'FAVYYCHLS' (SEQ ID NO:75) found in the Framework 3/LCDR-3 of hu0301 was eliminated in all lead clones by the non-conservative, germlining mutation H>Q at position 7. Similarly, for all clones other than 30E06, the TCED+ and LAF peptide 'IYAASNLES' (SEQ ID NO:76) which spans the Framework 2/LCDR-2 sequence was ablated by the germlining mutation L>R at position 7. Surprisingly, the stabilising mutations found in the LCDR-1 of all lead clones (replacing aspartic acid residues, which are mutations away from the IGKV1-16 germline), were not found to encode any GE, HAF or LAF epitopes in that region. The mutation and reselection process had therefore successfully identified non-germline, non-murine residues in the LCDR-1 that minimised development risk, maintained potency and did not raise immunogenicity risk. In the VH region of hu0301, the peptide sequence 'FKGRVTITA' (SEQ ID NO:77) (spanning the HCDR-2 and Framework 3 regions) was found to be a LAF. The germlining mutation K>Q at position 2, found in all leads (Table 4), eliminated this risk and converted the peptide sequence into a GE.

As the library selection and screening process had identified multiple novel CDR sequences (Table 3) and the analyses of these sequences had allowed the definition of regions of mutagenic tolerance in the CDRs (Fig. 2A, 2B), we hypothesised that *in silico* exploratory mutagenesis of the CDR regions of the key lead clone MH5 could potentially identify novel

derivatives of that clone which might retain its high functionality and further lower its immunogenicity risk. In the VH domain of clone MH5, a HAF epitope was found to be encoded by a 9-mer peptide beginning at position Y32 (Kabat numbering scheme) in the HCDR1. As this position was known to be highly tolerant of mutation (Fig. 2B), and *in silico* mutagenesis at this position demonstrated that the HAF could potentially be ablated (Table 8), it was chosen as a position to be sampled experimentally (Table 11). Also in the VH domain of clone MH5, LAF and HAF epitopes were found to be encoded at positions F99 and L100B respectively (Kabat numbering scheme), in the HCDR3/FW4 regions. As position F99 was not previously found to be tolerant of mutation (Fig. 2B), and *in silico* mutagenesis at position Q105 demonstrated that the higher-risk HAF at position L100B could potentially be ablated (Table 9), only Q105 was chosen as a position to be sampled experimentally (Table 11). Finally, In the VL domain of clone MH5, HAF epitopes were found to be encoded by 9-mer peptides beginning at position L46 and L47 (Kabat numbering scheme) in the FW2/LCDR2 region. As nothing was known about the tolerance of either of these positions to mutation, *in silico* mutagenesis at these positions and across both full 9-mers was undertaken. This analysis demonstrated that the HAF epitopes could potentially be ablated (Table 10), with positions L46, L47 and N53 being chosen as positions to be sampled experimentally (Table 11).

20 **Deimmunized MH5 derivatives - IgG specificity and potency characteristics**

The designer, MH5-derived, deimmunization mutant VH and VL domains designs described in Table 11 were gene synthesised, cloned in human IgG4-S228P expression vector format and combined for expression and purification in the matrix outlined in Table 12. These IgGs had minimised predicted T cell epitope content, as outlined in Table 13. Purified IgGs described above were then tested for binding to human and cyno CSF1R-Fc in direct titration ELISA format (Fig. 6A, 6B). This analysis demonstrated that all clones had retained human and cyno CSF1R binding profiles, but a subset of clones demonstrated reduced binding to cyno CSF1R (Fig. 6A, 6B). Antibodies that demonstrated fully retained binding to both orthologs of CSF1R were analysed for concentration-dependent binding at the cell surface via flow cytometry on HEK-293 cells transiently-transfected with human CSF1R. All clones analysed exhibited strong concentration-dependent binding to membrane-presented human CSF1R with binding curves overlapping with mu0301 and hu0301 IgGs (Fig. 7). No binding signals were observed for any clone on untransfected HEK-293 cells. Additionally, despite the significant additional mutagenesis in all clones, no binding above background was observed in 'PK-risk indicator' ELISA assays on human insulin and dsDNA, which examine the potential for polyreactivity (Fig. 8). All clones showed signals not only below the positive

control antibodies Bococizumab and Briakinumab, but also below the negative control antibodies Ustekinumab and Bevacizumab.

Biacore analyses of binding affinity were performed for all high-performing IgGs to solution-phase, monomeric human and cyno CSF1R proteins (Table 13). These analyses showed that heavily deimmunized clones which performed best in ELISA and FACS analyses also showed highest affinity binding to human and cyno CSF1R. Importantly, multiple clones (such as MH12, MH13, MH24 and MH16) maintained affinity values within 2-fold of hu0301 for both human and cyno CSF1R and retained human/cyno differentials of < 3-fold. Additionally, maximally deimmunized clones MH29, MH32 and MH40 still maintained affinities in the sub-nM range for both orthologs of CSF1R, proving that high potency, low immunogenicity and improved developability characteristics could be fully optimised in single clones (Table 14). The observation that some clones showed significantly reduced affinities (into the nM range) also highlighted the difficulty in deciding '*a priori*' which CDR residues may or may not be mutated to human germline or non-germline identity, when attempting to maximise the deimmunisation level of individual clones.

Lead clones 30E06, MH5, MH12 and MH16 were further examined for biological potency in a human *ex-vivo* monocyte assay of cell proliferation driven by MCSF-CSF1R signalling (Fig. 9). In this assay, all clones were tested using monocytes derived from 3 separate human donors. In all donor analyses, clones hu0301, 30E06, MH5, MH12 and MH16 showed fully overlapping curves for blockade of MCSF-driven cell proliferation, indicating that full biological activity was retained in each example lead clone.

25 **IgG developability analyses**

Differential Scanning Calorimetry (DSC) is used to measure thermal stability of proteins, as an indicator of overall molecular structural stability. This method was applied to clones hu0301, 30E06, MH5, MH12 and MH16, all in IgG4(S228P) format and formulated at 10mg/ml concentration into a standard sample buffer (PBS, 100mM L-Arginine). All IgGs tested were fully compatible with DSC analysis, presenting comparable sample homogeneity and cooperativity (Fig. 10). The measured thermal transition midpoints (T_m) for each antibody are indicated in Table 15. All five IgGs demonstrated Fab domain T_m values above 66 °C, indicating that all samples had high integrity. Unexpectedly, however, Fab domain T_m values varied widely across lead IgGs, with clones MH5 and 30E06 demonstrating the highest stability in their Fab domains (T_m values 77.7 °C and 76.6 °C, respectively). hu0301, in contrast, had a significantly lower stability Fab T_m value of 72.6

°C (Table 15). The significant increases in Fab stability for the MH5 and 30E06 lead antibodies over hu0301 were unexpected as the variable domain framework regions and antibody constant regions of all antibodies were near identical in sequence and all improvements were therefore mediated solely by differences in CDR sequences. In

5 contrast, the heavily-deimmunized clones MH12 and MH16 exhibited significantly reduced thermal stability (Fab T_m 66.4 and 66.9, respectively). This finding illustrates the inability to define a priori which v-domain residues can be modified to remove t cell epitope risk, without increasing thermostability risk.

10 Oxidation of exposed amino acid residues, such as tryptophan and methionine, is a common degradation pathway for mAbs. Importantly, oxidation of critical side chains in the CDRs of antibodies can also potentially impact on their biological activity, by causing a reduction in target binding affinity. Oxidation is a process that usually happens over time in the storage of proteins, so the standard laboratory method of analysing oxidative risk in

15 real time is to add an oxidative reagent to the protein. In this study, forced oxidation was applied to the IgGs by treating with 0.5 % H₂O₂ in PBS, for 2 hours at room temperature. As oxidation can alter overall hydrophobicity of an antibody, for example by increasing the polarity of the oxidised form, potential changes induced by forced oxidation were analysed by Reverse Phase (RP) Chromatography of tryptic digest peptides. RP analysis of tryptic

20 peptide fingerprints before and after H₂O₂ treatment showed that side chain oxidation changes were unexpectedly pronounced for clone hu0301. For hu0301, forced oxidation led to the modification of 8 peptides (Fig. 11A). In contrast, clones 30E06, MH5, MH12 and MH16 showed significantly fewer peptide modifications. For example, MH5 exhibited only 3 peptide modifications, all of which were minor (Fig. 11B). Clones 30E06, MH12 and MH16

25 exhibited 4, 4 and 5 modifications, respectively (Fig 11C, D, E). The improvements in oxidation degradation potential observed in all of clones 30E06, MH5, MH12 and MH16, in comparison to hu0301, was unexpected as none of the lead clones had altered content of oxidation-sensitive residues in either their CDRs or v-gene framework regions in comparison to hu0301 (Tables 2, 4, 11). Indeed, as clones MH12 and MH16 were actually

30 found to be less thermostable than hu0301 (Fig. 10), these improvements were not associated with improvements in total v-gene stability, but rather by unpredictable changes in the exposure of oxidation-sensitive side chains.

To examine the unique characteristics of the lead antibodies further, whole-IgG HIC

35 chromatography analyses were performed. HIC chromatograms showed that all 5 lead clones eluted in the same overall range as a group of control antibodies marketed for clinical

use (Fig. 12), but multiple peaks were observed for clones hu0301 (3 large and 3 small peaks, widely distributed), MH12 and MH16 (3 distinct peaks). In contrast, clones MH5 and 30E06 exhibited more uniform, single peaks (Fig 12), indicating that the expressed proteins exhibit lower structural heterogeneity.

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The combined analyses outlined herein demonstrated that, surprisingly, deep sampling of both germline and non-germline amino acids in multiple CDRs of these antibodies allowed the simultaneous optimisation of target binding specificity, immunogenicity risk, potency, biophysical stability and chemical stability risks in multiple final molecules.

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Although the present invention has been described with reference to preferred or exemplary embodiments, those skilled in the art will recognize that various modifications and variations to the same can be accomplished without departing from the spirit and scope of the present invention and that such modifications are clearly contemplated herein. No limitation with respect to the specific embodiments disclosed herein and set forth in the appended claims is intended nor should any be inferred.

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Table 1. Amino acid sequences murine anti-CSF1R CDRs as defined here ("Unified" scheme) in comparison to alternative definitions.

<u>Scheme</u>	<u>HCDR1</u>	<u>HCDR2</u>	<u>HCDR3</u>	<u>LCDR1</u>	<u>LCDR2</u>	<u>LCDR3</u>
Unified	GYTFTDNYMI (SEQ ID NO:78)	IGDINPYNGGTTFNQKFKG (SEQ ID NO:83)	ESPYFSNLYVMDY (SEQ ID NO:90)	KASQSVDDYDGDNYMN (SEQ ID NO:94)	AASNLES (SEQ ID NO:98)	HLSNEDLST (SEQ ID NO:100)
Kabat	DNYMI (SEQ ID NO:79)	DINPYNGGTTFNQKFKG (SEQ ID NO:84)	ESPYFSNLYVMDY (SEQ ID NO:90)	KASQSVDDYDGDNYMN (SEQ ID NO:94)	AASNLES (SEQ ID NO:98)	HLSNEDLST (SEQ ID NO:100)
Chotia	GYTFTDN (SEQ ID NO:80)	NPYNGG (SEQ ID NO:85)	ESPYFSNLYVMDY (SEQ ID NO:90)	KASQSVDDYDGDNYMN (SEQ ID NO:94)	AASNLES (SEQ ID NO:98)	HLSNEDLST (SEQ ID NO:100)
IMGT	GYTFTDNYM (SEQ ID NO:81)	INPYNGG (SEQ ID NO:86)	ARESPYFSNLYVMDY (SEQ ID NO:91)	QSVDDYDGDNY (SEQ ID NO:95)	AAS (SEQ ID NO:100)	HLSNEDLST (SEQ ID NO:100)
AHo	GYTFTDNYMI (SEQ ID NO:78)	INPYNGGTTFNQKFKG (SEQ ID NO:87)	ESPYFSNLYVMD (SEQ ID NO:92)	ASQSVDDYDGDNY (SEQ ID NO:96)	AASNLES (SEQ ID NO:98)	SNEDLS (SEQ ID NO:101)
AbM	GYTFTDNYMI (SEQ ID NO:78)	DINPYNGGT (SEQ ID NO:88)	ESPYFSNLYVMDY (SEQ ID NO:90)	KASQSVDDYDGDNYMN (SEQ ID NO:94)	AASNLES (SEQ ID NO:98)	HLSNEDLST (SEQ ID NO:100)
Contact	TDNYMI (SEQ ID NO:82)	IGDINPYNGGT (SEQ ID NO:89)	ARESPYFSNLYVMDY (SEQ ID NO:93)	DYDGDNYMNWY (SEQ ID NO:97)	LLIYAASNLE (SEQ ID NO:99)	HLSNEDLS (SEQ ID NO:102)

Table 2. Amino acid sequence of murine 0301 anti-CSF1R (mu0301), humanized (hu0301) and germline grafted v-domains.

V DOMAIN	Human germline¹	Amino acid sequence²
Mu0301-VH	n/a	EVQLQSGPELVRPGASVKMSCKAS <u>GYTF</u> TDNYMIWVKQSHGKSLEW <u>IGDINP YNGGTTFFNQKFKG</u> KATLTVEKSSSTAYMQLNSLTSED SAVYVCAR <u>ESPYFSNLYVMDY</u> WGQGSTVTVSS (SEQ ID NO:103)
Hu0301-VH	IGHV1-69 ³	QVQLVQSGAEVKPKGSSVKVSCCKAS <u>GYTF</u> TDNYMIWVRQAPGGGLEW <u>MGDINP YNGGTTFFNQKFKG</u> KRVITITAD <u>K</u> STSTAYMELSSLRSEDTAVYVCAR <u>ESPYFSNLYVMDY</u> WGQGSTLTIVSS (SEQ ID NO:104)
VH-graft ⁴	IGHV1-69	QVQLVQSGAEVKPKGSSVKVSCCKAS <u>GYTF</u> TDNYMIWVRQAPGGGLEW <u>MGDINP YNGGTTFFNQKFKG</u> KRVITITADESTSTAYMELSSLRSEDTAVYVCAR <u>ESPYFSNLYVMDY</u> WGQGSTLTIVSS (SEQ ID NO:105)
Mu0301-VL	n/a	NIVLTQSPASLAVSLGQRATIS <u>KASQSYDYDGDNTYMN</u> WYQKPGQPPKLLIY <u>AASNLES</u> GIPARFSGSGGTDFTLNIHPVEEDAATY <u>C</u> <u>HLSNEDLS</u> TFGGGTKLEIK (SEQ ID NO:106)
Hu0301-VL	IGKV3-11	EIVLTQSPATLSLSPGERATLS <u>KASQSYDYDGDNTYMN</u> WYQKPGQAPRLLIY <u>AASNLES</u> GIPARFSGSGGTDFTLTISSLEPEDFAVY <u>C</u> <u>HLSNEDLS</u> TFGGGTKVEIK (SEQ ID NO:107)
VL-graft ⁴	IGKV3-11	EIVLTQSPATLSLSPGERATLS <u>RASQSYDYDGDNTYMN</u> WYQKPGQAPRLLIY <u>AASNLES</u> GIPARFSGSGGTDFTLTISSLEPEDFAVY <u>C</u> <u>HLSNEDLS</u> TFGGGTKVEIK (SEQ ID NO:108)

¹Human germline definitions used for grafting, based on IMGT system. ²CDR residues are in bold and underlined. ³Sequence contains a residue 'K' in the framework, bold, italicised, that indicates a non-IGHV1-69*01 allelic variant of IGHV1-69. ⁴Germline grafts used for library construction, including the IGHV1-69*01 VH germline which was used for grafting and library construction. As noted above, the "Unified" CDR definitions used in this manuscript are an expanded definition in comparison to the classical Kabat definition. Each sequence above shows the framework regions (FRs) and the CDRs in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

Table 3. Unique CDRs from Fab clones shown to bind human and cyno CSF1R proteins.

<u>LCDR1</u>	<u>LCDR2</u>	<u>LCDR3</u>	<u>HCDR1</u>	<u>HCDR2</u>	<u>HCDR3</u>
RASQSVSYDGENYLN (SEQ ID NO:109)	AASNLET (SEQ ID NO:138)	QLSNQDLLT (SEQ ID NO:36)	GYTFSSYYMI (SEQ ID NO:31)	MGDINPYNGGTTTAAQKFQG (SEQ ID NO:42)	EGPYFGNLYVMDY (SEQ ID NO:227)
RASQSVEYEGDNYLN (SEQ ID NO:49)	AASNRET (SEQ ID NO:38)	HLSNEDLLT (SEQ ID NO:144)	GYTFSSYYMI (SEQ ID NO:41)	MGDINPYNGTTTFAQKFQG (SEQ ID NO:196)	ESDYFSNLYVMDY (SEQ ID NO:228)
RASQSVSYEGDNYLN (SEQ ID NO:110)	AASNLET (SEQ ID NO:43)	QLSNEDLLT (SEQ ID NO:39)	GYTFSSNYMI (SEQ ID NO:50)	MGDINPYNGGTTYNQKFQG (SEQ ID NO:197)	EVPIFSNLYVMDY (SEQ ID NO:229)
RASQSVEYDGENYLN (SEQ ID NO:111)	AASNRET (SEQ ID NO:139)	QLSNEWLLT (SEQ ID NO:145)	GYTFSSNYMI (SEQ ID NO:164)	MGDINPYNGTATYAAQKFQG (SEQ ID NO:198)	EGPYFSNLYVMDY (SEQ ID NO:33)
RASQSVSYEGENYLA (SEQ ID NO:40)	DASNRET (SEQ ID NO:35)	QLSSEDLLT (SEQ ID NO:56)	GYTFSDYYMI (SEQ ID NO:165)	MGDINPYNGGTTFAQKFQG (SEQ ID NO:199)	EDPYFSNLYVMDY (SEQ ID NO:230)
RASQSVEYQGDNYLA (SEQ ID NO:112)	DASNLET (SEQ ID NO:140)	QLSNEDLLT (SEQ ID NO:146)	GYTFSDNYMI (SEQ ID NO:166)	MGDINPYNGGTNFAQKFQG (SEQ ID NO:200)	EPPIFSNLYVMDY (SEQ ID NO:48)
RASQSVSYEGENYLN (SEQ ID NO:113)	DASNRET (SEQ ID NO:141)	QLSSEWLLT (SEQ ID NO:147)	GYTFSSNYII (SEQ ID NO:167)	MGDINPYNGGANFAQKFQG (SEQ ID NO:41)	ESPYTSNLYVMDY (SEQ ID NO:231)
RASQSVSYEGENYLN (SEQ ID NO:37)	DASNLET (SEQ ID NO:142)	QLSNNDLLT (SEQ ID NO:148)	GYTFSDNYII (SEQ ID NO:168)	MGDINPYNGGANYAAQKFQG (SEQ ID NO:32)	EPPIFRNLYVMDY (SEQ ID NO:232)
RASQSVEYQGDNYLN (SEQ ID NO:45)	GASNRET (SEQ ID NO:143)	QLSSQDLLT (SEQ ID NO:149)	GYTFSDNYII (SEQ ID NO:169)	MGDINPYNGGTTFNQKFQG (SEQ ID NO:201)	ESPYDSNLYVMDY (SEQ ID NO:233)
RASQSVEYQGENYLN (SEQ ID NO:53)		HLSNQDLLT (SEQ ID NO:150)	GGTFSSNYII (SEQ ID NO:170)	MGDINPYNGGATFAQKFQG (SEQ ID NO:202)	ETPIFSNLYVMDY (SEQ ID NO:234)
RASQSVSYDGDNYLN (SEQ ID NO:114)		QSNQDLLT (SEQ ID NO:151)	GYTFSDYYMI (SEQ ID NO:171)	MGDINPYNGTTTFNQKFQG (SEQ ID NO:203)	EHPIFSNLYVMDY (SEQ ID NO:235)
KASQSVSYDGDNYMN (SEQ ID NO:22)		QLSNEWLLT (SEQ ID NO:152)	GYTFSSYYII (SEQ ID NO:172)	MGDINPYNGGATYAAQKFQG (SEQ ID NO:47)	ESPYASNLYVMDY (SEQ ID NO:236)
RASQSVSYQGENYLN (SEQ ID NO:115)		QLSNQWLLT (SEQ ID NO:153)	GYTFSDYYIS (SEQ ID NO:173)	MGDINPYNGGANYNQKFQG (SEQ ID NO:204)	ESPIYSNLYVMDY (SEQ ID NO:237)
RASQSVSYDGDNYLN (SEQ ID NO:116)		NLSNEDLLT (SEQ ID NO:154)	GGTFSDYYMI (SEQ ID NO:174)	MGDINPYNGGTNYNQKFQG (SEQ ID NO:205)	EHPISSNLYVMDY (SEQ ID NO:238)
RASQSVSYQGDNYLN (SEQ ID NO:117)		QLSNQDLST (SEQ ID NO:155)	GYTFSSNYII (SEQ ID NO:54)	MGDINPYNGGTNFNQKFQG (SEQ ID NO:206)	ESAYFSNLYVMDY (SEQ ID NO:239)
RASQSVSYEGDNYLN (SEQ ID NO:118)		HLSNNDLLT (SEQ ID NO:156)	GYTFSSYYII (SEQ ID NO:46)	MGDINPYNGGATYNQKFQG (SEQ ID NO:51)	EGPIFRNLYVMDY (SEQ ID NO:240)
RASQSVSYQGENYLN (SEQ ID NO:119)		HLSNQDLST (SEQ ID NO:157)	GGTFSSYYMI (SEQ ID NO:175)	MGDINPYNGTANYNQKFQG (SEQ ID NO:207)	EGPIFENLYVMDY (SEQ ID NO:241)
RASQSVEYDGDNYLN (SEQ ID NO:120)		QSNEDLLT (SEQ ID NO:158)	GYTFSSYYIS (SEQ ID NO:176)	MGDINPYNGGTNYAAQKFQG (SEQ ID NO:55)	ESPIHSNLYVMDY (SEQ ID NO:242)
RASQSVSYDGDNYLN (SEQ ID NO:121)		QLSSEDLLT (SEQ ID NO:159)	GYTFSSYYMS (SEQ ID NO:177)	MGDINPYNGTANFNQKFQG (SEQ ID NO:208)	ESPIYSNLYVMDY (SEQ ID NO:243)
RASQSVSYDGENYLA (SEQ ID NO:122)		HQSNEDLLT (SEQ ID NO:160)	GYTFSSNYIS (SEQ ID NO:178)	MGDINPYFGGTTTAAQKFQG (SEQ ID NO:209)	ESPIYSNLYVMDY (SEQ ID NO:244)
RASQSVEYEGENYLN (SEQ ID NO:52)		QLSSNDLLT (SEQ ID NO:161)	GYTFSSNYMS (SEQ ID NO:179)	MGDINPYNGGATFNQKFQG (SEQ ID NO:210)	EGPKFSNLYVMDY (SEQ ID NO:245)
RASQSVEYDGDNYLA (SEQ ID NO:123)		QLSNNDLLT (SEQ ID NO:162)	GGTFSDNYMI (SEQ ID NO:180)	MGDINPYNGTANYAAQKFQG (SEQ ID NO:211)	ESLYFSNLYVMDY (SEQ ID NO:246)

RASQSVSYDGDNYLA (SEQ ID NO:124)		QQSSEDLT (SEQ ID NO:163)	GYTFSSYMS (SEQ ID NO:181)	MGDINPYNGTATYNQKFQG (SEQ ID NO:212)	EHPYFSQLYVMDY (SEQ ID NO:247)
RASQSVEYDGENYLA (SEQ ID NO:125)			GYTFDTDYII (SEQ ID NO:182)	MGDINPYNGTTTYNQKFQG (SEQ ID NO:213)	ESPYQSNLYVMDY (SEQ ID NO:248)
RASQSVEYQGENYLA (SEQ ID NO:126)			GYTFDTNYIS (SEQ ID NO:183)	MGDINPYNGTANFAQKFQG (SEQ ID NO:214)	ESEYFSNLYVMDY (SEQ ID NO:249)
RASQSVSDYQGDNYLN (SEQ ID NO:127)			GYTFDTNYMS (SEQ ID NO:184)	MGDINPYNGTTTYAQKFQG (SEQ ID NO:215)	ENPYFSNLYVMDY (SEQ ID NO:250)
RASQSVSYQGENYLA (SEQ ID NO:134)			GYTFSSYIS (SEQ ID NO:185)	MGDINPYNGGANFNQKFQG (SEQ ID NO:216)	EIPYFSNLYVMDY (SEQ ID NO:251)
RASQSVSDYDGENYLA (SEQ ID NO:128)			GYTFSTNYIS (SEQ ID NO:186)	MGDINPYNGTTNYAQKFQG (SEQ ID NO:217)	EPYFSNLYVIDY (SEQ ID NO:252)
RASQSVSYNGDNYLN (SEQ ID NO:129)			GYTFSDYIS (SEQ ID NO:187)	MGDINPYNGTATFAQKFQG (SEQ ID NO:218)	ESSYFSNLYVMDY (SEQ ID NO:253)
RASQSVEYHGDNYLN (SEQ ID NO:130)			GGTFDTYMI (SEQ ID NO:188)	MGDINPYNGTTNYNQKFQG (SEQ ID NO:219)	ESPYGSNLYVMDY (SEQ ID NO:254)
RASQSVSDYQGDNYLA (SEQ ID NO:131)			GYTFSDNYIS (SEQ ID NO:189)	MGDINPYFGGTTYNQKFQG (SEQ ID NO:220)	EPYLSNLYVMDY (SEQ ID NO:255)
RASQSVSDYDGDNYLA (SEQ ID NO:132)			GYTFDTDYMS (SEQ ID NO:190)	MGDINPYNGTATFNQKFQG (SEQ ID NO:221)	EPYFSNLYVADY (SEQ ID NO:256)
RASQSVEYEGDNYLA (SEQ ID NO:133)			GGTFDTNYII (SEQ ID NO:191)	MGDINPYNGTTNFNQKFQG (SEQ ID NO:222)	ESPYKSNLYVMDY (SEQ ID NO:257)
RASQSVSDYEGENYLA (SEQ ID NO:134)			GGTFSTNYII (SEQ ID NO:192)	MGDINPYFGGATYAQKFQG (SEQ ID NO:223)	EGPYRSNLYVMDY (SEQ ID NO:258)
RASQSVEYEGENYLA (SEQ ID NO:135)			GYTFSTNYMS (SEQ ID NO:193)	MGDINPYFGTTTYAQKFQG (SEQ ID NO:224)	ESPRFSNLYVMDY (SEQ ID NO:259)
RASQSVSDYEGDNYLA (SEQ ID NO:136)			GYTFSDYMS (SEQ ID NO:194)	MGDINPYFGGATYNQKFQG (SEQ ID NO:225)	ESPMFSNLYVMDY (SEQ ID NO:260)
RASQSVSDYQGENYLA (SEQ ID NO:137)			GGTFDTDYIS (SEQ ID NO:195)	MGDINPYNGTTNFAQKFQG (SEQ ID NO:226)	EYPYFSNLYVMDY (SEQ ID NO:261)
					EGPYQSNLYVMDY (SEQ ID NO:262)
					EDPYFSNLWVMDY (SEQ ID NO:263)
					ESPYFSNLYVMDY (SEQ ID NO:264)
					ESPPFSNLYVMDY (SEQ ID NO:265)
					EGPYLSNLYVMDY (SEQ ID NO:266)
					ESNYFSNLYVMDY (SEQ ID NO:267)
					EGPYYSNLYVMDY (SEQ ID NO:268)
					EGPYMSNLYVMDY (SEQ ID NO:269)

					ESPYFSGLYVMDY (SEQ ID NO:270)
					ERPYFSNLYVMDY (SEQ ID NO:271)
					ESTYFSNLYVMDY (SEQ ID NO:272)
					EGPYTSNLYVMDY (SEQ ID NO:273)
					EGPYDSNLYVMDY (SEQ ID NO:274)
					EPPYFGNLYVMDY (SEQ ID NO:275)
					EGPYFDNLYVMDY (SEQ ID NO:276)
					EPPYFSNSYVMDY (SEQ ID NO:277)
					EGPYFSNLYVMDY (SEQ ID NO:278)
					EGPYFSMLYVMDY (SEQ ID NO:279)
					EPPYFSNHYVMDY (SEQ ID NO:280)
					ESPYWSNLYVMDY (SEQ ID NO:281)
					EGPYESNLYVMDY (SEQ ID NO:282)
					EPPYFSELYVMDY (SEQ ID NO:283)
					EGPYSSNLYVMDY (SEQ ID NO:284)
					DSPYFSNLYVMDY (SEQ ID NO:285)

Table 4. CDR sequences of unique, library-derived and designer, CSF1R antagonistic IgGs.

<u>Clone name</u>	<u>LCDR1</u>	<u>LCDR2</u>	<u>LCDR3</u>	<u>HCDR1</u>	<u>HCDR2</u>	<u>HCDR3</u>
29D10	RASQSVSYEGE NYLN (SEQ ID NO:37)	AASNRAT (SEQ ID NO:38)	QLSNEDLLT (SEQ ID NO:39)	GYTFTSYMI (SEQ ID NO:41)	MGDINPYNGGANF AQKFQG (SEQ ID NO:41)	EGPYFSNLYV MDY (SEQ ID NO:33)
29B07	RASQSVSYEGD NYLN (SEQ ID NO:49)	AASNRAT (SEQ ID NO:38)	QLSNQDLLT (SEQ ID NO:36)	GYTFTSYII (SEQ ID NO:46)	MGDINPYNGGATY AQKFQG (SEQ ID NO:47)	EPPYFSNLYV MDY (SEQ ID NO:48)
30C11	RASQSVSYEGD NYLN (SEQ ID NO:110)	AASNLET (SEQ ID NO:138)	QLSNQDLLT (SEQ ID NO:36)	GYTFTSNYMI (SEQ ID NO:164)	MGDINPYNGTATY AQKFQG (SEQ ID NO:198)	EDPYFSNLYV MDY (SEQ ID NO:230)

26B07	RASQSVYQGD NYLN (SEQ ID NO:45)	AASNRAT (SEQ ID NO:38)	QLSNEDLLT (SEQ ID NO:39)	GYTFSSYYMI (SEQ ID NO:31)	MGDINPYNGGANY AQKFQG (SEQ ID NO:32)	EGPYFSNLYV MDY (SEQ ID NO:33)
29A03	RASQSVYEYEGE NYLN (SEQ ID NO:52)	AASNRAT (SEQ ID NO:38)	QLSNQDLLT (SEQ ID NO:36)	GYTFSSNYMI (SEQ ID NO:50)	MGDINPYNGGATY NQKFQG (SEQ ID NO:51)	EGPYFSNLYV MDY (SEQ ID NO:48)
29E11	RASQSVSYDGE NYLA (SEQ ID NO:122)	AASNRAT (SEQ ID NO:38)	QLSNEDLLT (SEQ ID NO:39)	GYTFTSYMYI (SEQ ID NO:41)	MGDINPYNGTATY AQKFQG (SEQ ID NO:198)	EGPYFSNLYV MDY (SEQ ID NO:33)
30G02	RASQSVSYQGE NYLN (SEQ ID NO:115)	AASNRAT (SEQ ID NO:38)	QLSSEDLLT (SEQ ID NO:56)	GYTFTSNYYI (SEQ ID NO:54)	MGDINPYNGGTTY AQKFQG (SEQ ID NO:55)	EGPYFSNLYV MDY (SEQ ID NO:33)
30E06	RASQSVSYEGE NYLA (SEQ ID NO:40)	AASNLAT (SEQ ID NO:43)	QLSNEDLLT (SEQ ID NO:39)	GYTFTSYMYI (SEQ ID NO:41)	MGDINPYNGGTTY AQKFQG (SEQ ID NO:42)	EGPYFSNLYV MDY (SEQ ID NO:33)
29H09	RASQSVSYEGE NYLN (SEQ ID NO:37)	AASNRAT (SEQ ID NO:38)	QLSNEDLLT (SEQ ID NO:39)	GYTFTSYMYI (SEQ ID NO:41)	MGDINPYNGGANY AQKFQG (SEQ ID NO:32)	EGPYFSNLYV MDY (SEQ ID NO:33)
30D02	RASQSVYQGE NYLN (SEQ ID NO:53)	AASNRAT (SEQ ID NO:38)	QLSNQDLLT (SEQ ID NO:36)	GYTFSSYYMI (SEQ ID NO:31)	MGDINPYNGGTTY AQKFQG (SEQ ID NO:42)	EGPYFSNLYV MDY (SEQ ID NO:33)
MH1	RASQSVSYQGE NYLA (SEQ ID NO:34)	DASNRAT (SEQ ID NO:35)	QLSNQDLLT (SEQ ID NO:36)	GGTFSSYYII (SEQ ID NO:286)	MGDINPYNGTANY AQKFQG (SEQ ID NO:211)	EGPYFSNLYV MDY (SEQ ID NO:33)
MH2	RASQSVSYEGE NYLN (SEQ ID NO:37)	AASNRAT (SEQ ID NO:38)	QLSNEDLLT (SEQ ID NO:39)	GGTFSSYYII (SEQ ID NO:286)	MGDINPYNGTANY AQKFQG (SEQ ID NO:211)	EGPYFSNLYV MDY (SEQ ID NO:33)
MH3	RASQSVSYEGE NYLA (SEQ ID NO:40)	AASNRAT (SEQ ID NO:38)	QLSNQDLLT (SEQ ID NO:36)	GGTFSSYYII (SEQ ID NO:286)	MGDINPYNGTANY AQKFQG (SEQ ID NO:211)	EGPYFSNLYV MDY (SEQ ID NO:33)
MH4	RASQSVSYQGE NYLA (SEQ ID NO:34)	DASNRAT (SEQ ID NO:35)	QLSNQDLLT (SEQ ID NO:36)	GYTFSSYYMI (SEQ ID NO:31)	MGDINPYNGGANY AQKFQG (SEQ ID NO:32)	EGPYFSNLYV MDY (SEQ ID NO:33)
MH5	RASQSVSYEGE NYLN (SEQ ID NO:37)	AASNRAT (SEQ ID NO:38)	QLSNEDLLT (SEQ ID NO:39)	GYTFSSYYMI (SEQ ID NO:31)	MGDINPYNGGANY AQKFQG (SEQ ID NO:32)	EGPYFSNLYV MDY (SEQ ID NO:33)
MH6	RASQSVSYEGE NYLA (SEQ ID NO:40)	AASNRAT (SEQ ID NO:38)	QLSNQDLLT (SEQ ID NO:36)	GYTFSSYYMI (SEQ ID NO:31)	MGDINPYNGGANY AQKFQG (SEQ ID NO:32)	EGPYFSNLYV MDY (SEQ ID NO:33)
MH7	RASQSVSYQGE NYLA (SEQ ID NO:34)	DASNRAT (SEQ ID NO:35)	QLSNQDLLT (SEQ ID NO:36)	GYTFSSYYII (SEQ ID NO:172)	MGDINPYNGTANY AQKFQG (SEQ ID NO:211)	EGPYFSNLYV MDY (SEQ ID NO:33)
MH8	RASQSVSYEGE NYLN (SEQ ID NO:37)	AASNRAT (SEQ ID NO:38)	QLSNEDLLT (SEQ ID NO:39)	GYTFSSYYII (SEQ ID NO:172)	MGDINPYNGTANY AQKFQG (SEQ ID NO:211)	EGPYFSNLYV MDY (SEQ ID NO:33)
MH9	RASQSVSYEGE NYLA (SEQ ID NO:40)	AASNRAT (SEQ ID NO:38)	QLSNQDLLT (SEQ ID NO:36)	GYTFSSYYII (SEQ ID NO:172)	MGDINPYNGTANY AQKFQG (SEQ ID NO:211)	EGPYFSNLYV MDY (SEQ ID NO:33)

Table 5. Alphascreen IC50 values of anti-CSF1R IgGs.

<u>Clone ID</u>	<u>IC50 nM</u>
mu0301	1.29
hu0301	2.38
30E06	1.53
29D10	1.93
29H09	2.16
MH5	2.18
26B07	2.56
29B07	3.86
30D02	3.92
29A03	4.60
MH6	7.74
MH4	18.94

5

10

Table 6. Biacore affinity values for IgG binding to human and cyno monomeric CSF1R.

Clone name	<u>Human CSF1R</u>			<u>Cyno CSF1R</u>		
	Ka	Kd	KD	ka	kd	KD
	(1/Ms)	(1/s)	(nM)	(1/Ms)	(1/s)	(nM)
mu0301	2.90E+06	6.90E-05	0.024	7.40E+05	7.40E-05	0.1
hu0301	2.30E+06	4.70E-04	0.21	5.00E+05	3.30E-04	0.66
30E06	1.90E+06	1.90E-04	0.11	5.70E+05	1.40E-04	0.25
29H09	1.80E+06	3.40E-04	0.19	5.50E+05	2.30E-04	0.42
29D10	1.90E+06	3.70E-04	0.2	5.20E+05	2.20E-04	0.42
MH5	1.70E+06	3.60E-04	0.22	5.00E+05	3.10E-04	0.62
26B07	1.50E+06	4.20E-04	0.29	3.10E+05	2.60E-04	0.85
29B07	1.50E+06	5.10E-04	0.34	3.10E+05	2.50E-04	0.79
29A03	1.60E+06	6.00E-04	0.39	2.70E+05	3.20E-04	1.2
30D02	1.00E+06	4.90E-04	0.48	1.90E+05	3.20E-04	1.7
MH6	1.30E+06	1.20E-03	0.88	5.10E+05	6.40E-04	1.3
MH4	1.30E+06	2.40E-03	1.9	4.70E+05	1.40E-03	2.9
30G02	1.20E+06	8.80E-03	7.2	4.70E+05	6.20E-03	13.2

Table 7. Human T cell epitope content in v-domains predicted by iTOPE™ and TCED™.

Clone Name	Germline epitopes	Low Affinity Foreign	High Affinity Foreign	TCED+
hu0301	8	4	3	2
30E06	9	2	4	2
29H09	9	1	4	1
29D10	9	1	4	1
MH5	9	1	4	1
26B07	9	1	4	1
29B07	9	2	4	1
29A03	9	1	3	0
30D02	9	2	4	1
MH6	9	2	4	1
MH4	11	2	2	1
30G02	9	2	3	0

Table 8. The effect on iTope™ scores of proposed deimmunising changes for the antibody MH5 associated with VH domain epitope with a p1 anchor at Y32 (Kabat numbering scheme).

p1 Anchor	Sequence	MHC II Ligands	High Affinity Ligands
Y32	Y YMIWVRQA	20	19
	A *****	0	0
	D *****	0	0
	G *****	0	0
	H *****	0	0
	N *****	0	0
	S *****	0	0
	T *****	0	0
	* D *****	18	10
	*** G *****	18	14
	*** H *****	20 ^Δ	17
	*** N *****	18 ^{ΔΔ}	17
	*** S *****	19 ^{ΔΔ}	19
	*** T *****	19 ^{ΔΔ}	17
	*** W *****	20	14
	*** Y *****	20	15

- 5 Dark grey cells indicates the presence of a High Affinity Foreign T cell epitope and light grey indicates the presence of a Low Affinity Foreign T cell epitope. ^Δ indicates that the core 9mer peptide is encoded by germline sequence. ^{ΔΔ} indicates that the core 9mer peptide is encoded by a TCED™ negative peptide. SEQ ID NOs are assigned to the sequences as shown below:

Sequence	SEQ ID NO
YYMIWVRQA	287
AYMIWVRQA	288
DYMIWVRQA	289
GYMIWVRQA	290
HYMIWVRQA	291
NYMIWVRQA	292
SYMIWVRQA	293
TYMIWVRQA	294
YDMIWVRQA	295

YYMGWVRQA	296
YYMHWVRQA	297
YYMNWVRQA	298
YYMSWVRQA	299
YYMTWVRQA	300
YYMWWVRQA	301
YYMYWVRQA	302

Table 9. The effect on iTope™ scores of proposed deimmunising changes for the antibody MH5 associated with the VH domain epitopes with p1 anchors at positions F99 and L100B (Kabat numbering scheme).

p1 Anchor	Sequence	MHC II Ligands	High Affinity Ligands	p1 Anchor	Sequence	MHC II Ligands	High Affinity Ligands
F99	FSNLYVMDYWGQ	19	16	L100B	FSNLYVMDYWGQ	25	17
	A*****	0*	0		A*****	25*	17
	H*****	0*	0		H*****	25*	17
	T*****	0*	0		T*****	25*	17
	D**	10	7		***D*****	0	0
	G**	12 ^a	8		***G*****	0 ^a	0
	H**	16	11		***H*****	0	0
	P**	9	3		***P*****	0	0
	Q**	15	12		***Q*****	0	0
	R**	12	9		***R*****	0	0
	S**	14	10		***S*****	0	0
	T**	14	8		***T*****	0	0
	W**	15	10		***W*****	16	10
	Y**	16	12		***Y*****	16	10
	****D*****	19**	16		****D*****	1**	0
	****S*****	19**	16		****S*****	15**	1
	****W*****	19**	16		****W*****	16**	3
	*****D*****	6	0		*****D*****	6	0
	*****D*****	8	1		*****D*****	5	3
	*****E*****	13	6		*****E*****	2	0
	*****G*****	14	7		*****G*****	7	6
	*****H*****	16	13		*****H*****	9	5
	*****P*****	16	10		*****P*****	1	0
	*****S*****	16	9		*****S*****	5	0
	*****T*****	15	9		*****T*****	6	1
	*****W*****	16	10		*****W*****	12	4
	*****K*****	19**	16		*****K*****	15**	4
	*****R*****	19**	16		*****R*****	12**	4

- 5 Dark grey cells indicates the presence of a High Affinity Foreign T cell epitope and light grey indicates the presence of a Low Affinity Foreign T cell epitope. * Changes at this position affect the MHC ligand with p1 anchor at F99 only. ** Changes at this position affect the MHC ligand with p1 anchor at L100B only. ^a Mutation using glycine in this position was discounted to avoid introducing a potential deamidation site. SEQ ID NOs are assigned to the sequences as shown below:
- 10

Sequence	SEQ ID NO
FSNLYVMDYWGQ	303
ASNLYVMDYWGQ	304
HSNLYVMDYWGQ	305
TSNLYVMDYWGQ	306
FSNDYVMDYWGQ	307
FSNGYVMDYWGQ	308
FSNRYVMDYWGQ	309
FSNSYVMDYWGQ	310
FSNTYVMDYWGQ	311
FSNWYVMDYWGQ	312
FSNYYVMDYWGQ	313

FSNLDVMDYWGQ	314
FSNLSVMDYWGQ	315
FSNLWVMDYWGQ	316
FSNLYDMDYWGQ	317
FSNLYVDDYWGQ	318
FSNLYVEDYWGQ	319
FSNLYVGDYWGQ	320
FSNLYVHDYWGQ	321
FSNLYVPDYWGQ	322
FSNLYVSDYWGQ	323
FSNLYVTDYWGQ	324
FSNLYVWDYWGQ	325
FSNLYVMDYWGK	326
FSNLYVMDYWGR	327

Table 10. The effect on iTope™ scores of proposed deimmunising changes for the antibody MH5 associated with the VL domain epitopes with p1 anchors at positions L46 and L47 (Kabat numbering scheme).

p1 Anchor	Sequence	MHC II Ligands	High Affinity Ligands	p1 Anchor	Sequence	MHC II Ligands	High Affinity Ligands
L46	LLIYAASNRA	28	17	L47	LLIYAASNRA	31	24
	A*****	0*	0		A*****	31*	24
	R*****	0*	0		R*****	31*	24
	S*****	0*	0		S*****	31*	24
	T*****	0*	0		T*****	31*	24
	*A*****	17	10		*A*****	0	0
	L***	24	13		**L*****	31	22
	H**	26	16		***H*****	26	16
	K**	26	16		***K*****	24	11
	S**	23	14		***S*****	26	16
	****D*****	28**Δ	17		****D*****	23**Δ	14
	****E*****	28**	17		****E*****	20**	11
	****T*****	28**	17		****T*****	25**	16
	*****D*****	9*	4		*****D*****	31*	24
	*****G*****	17*	12		*****G*****	31*	24
	*****D***	17	9		*****D***	10	7
	*****G***	23	16		*****G***	26	12
	*****D**	28**	17		*****D**	15**	8
	*****E**	28**	17		*****E**	24**	12
	*****D	28**	17		*****D	21**	14
	*****E	28**	17		*****E	23**	16
	*****G	28**	17		*****G	31**	16
	*****P	28**	17		*****P	22**	10

5 Dark grey cells indicates the presence of a High Affinity Foreign T cell epitope and light grey indicates the presence of a Low Affinity Foreign T cell epitope. *Changes at this position affect the MHC ligand with p1 anchor at L46 only. **Changes at this position affect the MHC ligand with p1 anchor at L47 only. ΔIndicates the core 9mer peptide is encoded by germline sequence. SEQ ID NOs are assigned to the sequences as shown below:

10

Sequence	SEQ ID NO
LLIYAASNRA	0328
ALIYAASNRA	0329
RLIYAASNRA	0330
SLIYAASNRA	0331
TLIYAASNRA	0332
LAIYAASNRA	0333
LLLYAASNRA	0334
LLIHAASNRA	0335
LLIKAASNRA	0336
LLISAASNRA	0337
LLIYDASNRA	0338
LLIYEASNRA	0339
LLIYTASNRA	0340

LLIYADSNRA	0341
LLIYAGSNRA	0342
LLIYAADNRA	0343
LLIYAAGNRA	0344
LLIYAASDRA	0345
LLIYAASERA	0346
LLIYAASNRD	0347
LLIYAASNRE	0348
LLIYAASNRG	0349
LLIYAASNRP	0350

Table 11. V-domain sequences of unique, designer, deimmunized, CSF1R-antagonistic IgGs.

MB-5.1 VH	QVQLVQSGAEVKKPGSSVKVCKKAS GYTFSS <u>NYMI</u> WVRQAPGQGLEWMGDI NPYNGG ANYAQAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARE GPYFS NLYVMDYWGQGTLVTVSS
MB-5.2 VH	QVQLVQSGAEVKKPGSSVKVCKKAS GYTFSS <u>AYMI</u> WVRQAPGQGLEWMGDI NPYNGG ANYAQAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARE GPYFS NLYVMDYWGQGTLVTVSS
MB-5.3 VH	QVQLVQSGAEVKKPGSSVKVCKKAS GYTFSS <u>SYMI</u> WVRQAPGQGLEWMGDI NPYNGG ANYAQAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARE GPYFS NLYVMDYWGQGTLVTVSS
MB-5.4 VH	QVQLVQSGAEVKKPGSSVKVCKKAS GYTFSS <u>HYMI</u> WVRQAPGQGLEWMGDI NPYNGG ANYAQAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARE GPYFS NLYVMDYWGQGTLVTVSS
MB-5.5 VH	QVQLVQSGAEVKKPGSSVKVCKKAS GYTFSS <u>NYMI</u> WVRQAPGQGLEWMGDI NPYNGG ANYAQAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARE GPYFS NLYVMDYWGQGTLVTVSS
MB-5.6 VH	QVQLVQSGAEVKKPGSSVKVCKKAS GYTFSS <u>AYMI</u> WVRQAPGQGLEWMGDI NPYNGG ANYAQAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARE GPYFS NLYVMDYWGQGTLVTVSS
MB-5.7 VH	QVQLVQSGAEVKKPGSSVKVCKKAS GYTFSS <u>SYMI</u> WVRQAPGQGLEWMGDI NPYNGG ANYAQAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARE GPYFS NLYVMDYWGQGTLVTVSS
MB-5.8 VH	QVQLVQSGAEVKKPGSSVKVCKKAS GYTFSS <u>HYMI</u> WVRQAPGQGLEWMGDI NPYNGG ANYAQAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARE GPYFS NLYVMDYWGQGTLVTVSS
MB-5.1 VL	EIVLTQSPATLSLSPGERATLSC RASQSVSYEGENY LNWYQQKPGQAPR SA IYAASNRRATGIPARESGSGSGTDFTLTLSPPEDFAVYYC QLSNED LLTTEGGGTVKEIK
MB-5.2 VL	EIVLTQSPATLSLSPGERATLSC RASQSVSYEGENY LNWYQQKPGQAPR AA IYAASNRRATGIPARESGSGSGTDFTLTLSPPEDFAVYYC QLSNED LLTTEGGGTVKEIK
MB-5.3 VL	EIVLTQSPATLSLSPGERATLSC RASQSVSYEGENY LNWYQQKPGQAPR SL IYAASDRATGIPARESGSGSGTDFTLTLSPPEDFAVYYC QLSNED LLTTEGGGTVKEIK
MB-Min VL	EIVLTQSPATLSLSPGERATLSC RASQSVSYEGENY LNWYQQKPGQAPRLLIYAASNRRATGIPARESGSGSGTDFTLTLSPPEDFAVYYC QLSNED LLTTEGGGTVKEIK

CDRs are in bold font. Deimmunizing mutations are in italic and underlined font. SEQ ID NOs are assigned to the V-domain sequences as shown below:

MH-5.1 VH (SEQ ID NO:1)	MH-5.1 VL (SEQ ID NO:9)
MH-5.2 VH (SEQ ID NO:2)	MH-5.2 VL (SEQ ID NO:10)
MH-5.3 VH (SEQ ID NO:3)	MH-5.3 VL (SEQ ID NO:11)
MH-5.4 VH (SEQ ID NO:4)	MH-Min VL (SEQ ID NO:12)
MH-5.5 VH (SEQ ID NO:5)	
MH-5.6 VH (SEQ ID NO:6)	
MH-5.7 VH (SEQ ID NO:7)	
MH-5.8 VH (SEQ ID NO:8)	

Table 12. V-domain sequence combinations of unique, designer, deimmunized, CSF1R-antagonistic IgGs.

Clone name	VH sequence	VL sequence
MH10	MH-5.1 VH	MH-5.1 VL
MH11		MH-5.2 VL
MH12		MH-5.3 VL
MH13		MH-Min VL
MH14	MH-5.2 VH	MH-5.1 VL
MH15		MH-5.2 VL
MH16		MH-5.3 VL
MH17		MH-Min VL
MH18	MH-5.3 VH	MH-5.1 VL
MH19		MH-5.2 VL
MH20		MH-5.3 VL
MH21		MH-Min VL
MH22	MH-5.4 VH	MH-5.1 VL
MH23		MH-5.2 VL
MH24		MH-5.3 VL
MH25		MH-Min VL
MH26	MH-5.5 VH	MH-5.1 VL
MH27		MH-5.2 VL
MH28		MH-5.3 VL
MH29		MH-Min VL
MH30	MH-5.6 VH	MH-5.1 VL
MH31		MH-5.2 VL
MH32		MH-5.3 VL
MH33		MH-Min VL
MH34	MH-5.7 VH	MH-5.1 VL
MH35		MH-5.2 VL
MH36		MH-5.3 VL
MH37		MH-Min VL
MH38	MH-5.8 VH	MH-5.1 VL
MH39		MH-5.2 VL
MH40		MH-5.3 VL
MH41		MH-Min VL

Table 13. Human T cell epitope content in v-domains predicted by iTOPE™ and TCED™.

Clone Name	Germline epitopes	Low Affinity Foreign	High Affinity Foreign	TCED+
hu0301	8	4	3	2
MH5	9	1	4	1
MH10, 11, 12	9	1	1	0
MH13	9	1	3	0
MH14, 15, 16	9	1	1	0
MH17	9	1	3	0
MH18, 19, 20	9	1	1	0
MH21	9	1	3	0
MH22, 23, 24	9	1	1	0
MH25	9	1	3	0
MH26, 27, 28	9	1	0	0
MH29	9	1	2	0
MH30, 31, 32	9	1	0	0
MH33	9	1	2	0
MH34, 35, 36	9	1	0	0
MH37	9	1	2	0
MH38, 39, 40	9	1	0	0
MH41	9	1	2	0

Table 14. Biacore affinity values for IgG4 binding to human and cyno monomeric CSF1R.

Clone name	<u>Human CSF1R</u>			<u>Cyno CSF1R</u>		
	Ka (1/Ms)	Kd (1/s)	KD (nM)	ka (1/Ms)	kd (1/s)	KD (nM)
mu0301	2.90E+06	6.90E-05	0.024	7.40E+05	7.40E-05	0.1
hu0301	2.30E+06	4.70E-04	0.21	5.00E+05	3.30E-04	0.66
MH5	1.70E+06	3.60E-04	0.22	5.00E+05	3.10E-04	0.62
MH12	2.43E+06	8.32E-04	0.342	1.30E+06	7.26E-04	0.557
MH13	1.69E+06	6.73E-04	0.397	8.27E+05	5.98E-04	0.723
MH24	2.46E+06	9.76E-04	0.397	1.07E+06	8.33E-04	0.777
MH16	2.02E+06	8.97E-04	0.444	9.56E+05	7.50E-04	0.785
MH40	2.05E+06	1.13E-03	0.553	1.06E+06	1.01E-03	0.948
MH29	1.71E+06	9.61E-04	0.562	8.91E+05	8.13E-04	0.913
MH32	2.04E+06	1.27E-03	0.62	1.05E+06	1.03E-03	0.979
MH10	1.41E+06	8.80E-04	0.624	7.05E+05	7.54E-04	1.07
MH25	1.45E+06	9.21E-04	0.634	7.33E+05	7.58E-04	1.03
MH20	2.49E+06	1.77E-03	0.709	1.08E+06	1.34E-03	1.24
MH11	1.53E+06	1.19E-03	0.776	8.02E+05	1.03E-03	1.29
MH17	1.35E+06	1.12E-03	0.834	6.83E+05	9.06E-04	1.33
MH41	1.28E+06	1.09E-03	0.851	6.66E+05	9.72E-04	1.46
MH33	1.32E+06	1.44E-03	1.09	6.60E+05	1.15E-03	1.74
MH21	1.54E+06	1.89E-03	1.23	7.46E+05	1.37E-03	1.84

Table 15. Thermal transition midpoints of selected clones in DSC.

<u>Clone ID</u>	<u>Fab Tm</u>	<u>CH2 Tm</u>
hu0301	72.60	69.5
30E06	76.60	69.5
MH5	77.70	69
MH12	66.40	na
MH16	66.90	na

na = transition obscured by Fab

Table 16. Examples of antibody variable region amino acid sequences.**Antibody MH5 light chain variable (VL) region**

5 EIVLTQSPATLSLSPGERATLSCRASQSVSYEGENYLNWYQQKPGQAPRLLIYAASNR
ATGIPARFSGSGSGTDFTLTISSPEPEDFAVYYCQLSNEDLLTFGGGTKVEIK (SEQ
ID NO:351)

Antibody MH5 heavy chain variable (VH) region

10 QVQLVQSGAEVKKPGSSVKVSCASGYTFSSYMIWVRQAPGQGLEWMGDINPYNGGA
NYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCAREGPYF SNLYVMDYWGQGT
LVTVSS (SEQ ID NO:352)

Antibody MH12 light chain variable (VL) region

15 QVQLVQSGAEVKKPGSSVKVSCASGYTFSSNYMIWVRQAPGQGLEWMGDINPYNGGA
NYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCAREGPYF SNLYVMDYWGQGT
LVTVSS (SEQ ID NO:353)

Antibody MH12 heavy chain variable (VH) region

20 EIVLTQSPATLSLSPGERATLSCRASQSVSYEGENYLNWYQQKPGQAPRSLIYAASDR
ATGIPARFSGSGSGTDFTLTISSPEPEDFAVYYCQLSNEDLLTFGGGTKVEIK (SEQ
ID NO:354)

Antibody MH16 light chain variable (VL) region

25 QVQLVQSGAEVKKPGSSVKVSCASGYTFSSAYMIWVRQAPGQGLEWMGDINPYNGGA
NYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCAREGPYF SNLYVMDYWGQGT
LVTVSS (SEQ ID NO:355)

Antibody MH16 heavy chain variable (VH) region

30 EIVLTQSPATLSLSPGERATLSCRASQSVSYEGENYLNWYQQKPGQAPRSLIYAASDR
ATGIPARFSGSGSGTDFTLTISSPEPEDFAVYYCQLSNEDLLTFGGGTKVEIK (SEQ
ID NO:356)

Antibody 30E06 light chain variable (VL) region

35 EIVLTQSPATLSLSPGERATLSCRASQSVSYEGENYLAWYQQKPGQAPRLLIYAASNL
ATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQLSNEDLLTFGGGTKVEIK (SEQ
ID NO:357)

Antibody 30E06 heavy chain variable (VH) region

40 QVQLVQSGAEVKKPGSSVKVSCASGYTFTSYMIWVRQAPGQGLEWMGDINPYNGGT
TYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCAREGPYF SNLYVMDYWGQGT
LVTVSS (SEQ ID NO:358)

Table 17. Examples of antibody Fc region amino acid sequences.**Human IgG4 wild type**

45 ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPSPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSDQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYCKVSNKGL
50 PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL
DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKS LSLSLGK (SEQ ID NO:359)

Human IgG4(S228P)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT
 CVVVDVSDQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGL
 5 PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL
 DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:360)

Human IgG1 wild type

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
 10 VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
 LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:361)

Human IgG1-3M

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE
 15 VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
 20 LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:362)

Human IgG1 wild type "REEM" allotype

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
 25 VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
 LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:363)

Human IgG1-3M "REEM" allotype

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE
 30 VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
 35 DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:364)

Table 18. Examples of CSFR1 amino acid sequences.

Human CSF1R sequence

MGPGVLLLLLLVATAWHGQGIPVIEPSVPELVVKPGATVTLRCVGNGSVVEWDGPPSPHWTL
 40 YSDGSSSILSTNNATFQNTGTYRCTEPGDPLGGSAAIHLYVKDPAWPWNVLAQEVVVFED
 QDALLPCLLTDPVLEAGVSLVRVRGRPLMRHTNYSFSPWHGFTIHRAKFIQSQDYQCSAL
 MGGRKVMSSIRLVKQVIPGPPALTLVPAELVRIRGEAAQIVCSASSVDVNFDFVLQHN
 NTKLAIPQQSDFHNNRYQKVLTLNLDQVDFQHAGNYSVASNVQGHSTSMFFRVVESAY
 45 LNLSSQNLIQEVTVGEGLNLKVMVEAYPGLQGFNWYTLGPFSDHQPEPKLANATTKDTY
 RHTFTLSLRLKPSEAGRYSFLARNPGGWRAITFELTLRYPPEVSVIWTFFINGSGTLLCA
 ASGYQPQNVTLQCSGHTDRCDQAQVLQVWDDPYPEVLSQEPFHKVTVQSLLTVETLEHN
 QTYECRAHNSVGSGSWAFIPISAGATHPPDEFLLTPVVVACMSIMALLLLLLLLLLLYKY
 KQPKYQVRWKIIESYEGNSYTFIDPTQLPYNEKWEFPRNNLQFGKTLGAGAFGKVVVEAT
 50 AFGLGKEDAVLVKAVKMLKSTAHADKEALMSELKIMSHLGQHENIVNLLGACTHGGPVL
 VITEYCCYGDLLNLFRRKAEAMLGSPSLSPGQDPEGGVVDYKNIHLEKKYVRRDSGFSQGV
 DTYVEMRPVSTSSNDSFSEQDLKEDGRPLELRDLLHFSSQVAQGMFLASKNCIHRDVA
 ARNVLLTNGHVAKIGDFGLARDIMNDSNYIVKGNARLPVKWMAPEISIFDCVYTVQSDVWS
 YGILLWEIFSLGLNPYPGILVNSKFYKLVKDGQMAQPAFAPKNIYSIMQACWALEPTHR
 55 PTFQQICSFLEQAQEDRRERDYNLPSSSRSGSGSSSSELEEESSEHLTCCEQGDIA
 QPLLQPNNYQFC (SEQ ID NO:365)

Cynomolgus monkey CSF1R sequence

MGPGVLLLLLLVATAWHGQGIPVIEPSGPELVVKPGETVTLRCVGNGSVVEWDGPISPHWTL
 60 YSDGPSSVLTNNATFQNTRTYRCTEPGDPLGGSAAIHLYVKDPAWPWNVLAKEVVVFED
 QDALLPCLLTDPVLEAGVSLVRLRGRPLLRHTNYSFSPWHGFIHRAKFIQGGDYQCSAL

MGGRKVMSSISIRLKVQKVIPGPPALTLPAPLVRIRGEAAQIVCSASNIDVDFDVFLQHN
TTKLAIPQRSDFHDNRYQKVLTLTSLGQVDFQHAGNYSCVASNVQGHSTSMFFRVVESAY
LDLSSEQNLIQEVTVGEGLNLKVMVEAYPGLQGFWNTYLGPFSDHQPEPKLANATTKDITY
RHTFTLSLPRLKPSEAGRYSFLARNPGGWRAITFELTLRYPPEVSVIWTSSINGSGTLLCA
5 ASGYPPQPNVTWLQCAGHTDRCDEAQVLQVWVDPHPEVLSQEPFQKVTVQSLLTAETLEHN
QTYECRAHNSVSGSGSWAFIPISAGARTHPPDEFLETPVVVACMSVMALLLLLLLLLLLYKY
KQKPKYQVRWKIIESYEGNSYTFIDPTQLPYNEKWEFPRNNLQFGKTLGAGAFGKVVEAT
AFGLGKEDAVLKVAVKMLKSTAHADKEALMSELKIMSHLGQHENIVNLLGACTHGGPVL
VITEYCCYGDLLNFLRRKAEAMLGPSLSPGQDPEGADYKNIHLEKKYVRRDSGFSSQGV
10 DTYVEMRPVSTSSNDSFSEQDLKDGRPLELWDLHFSSQVAQGMFLASKNCIHRDVA
ARNVLLTNGHVAKIGDFGLARDIMNDSNYIVKGNARLPVKWMAPEIFDCVYTVQSDVWS
YGILLWEIFSLGLNPYPGILVNSKFYKLVKDGYPMAQPAFAPKNIYSIMQACWALEPTHR
PTFQQICSLQEQAQEDRRERDYTNLPSSSRSGSGSGSSSSSSSEPEEESSSEHLACCEQ
GDIAQPLLQPNNYQFC (SEQ ID NO:366)

CLAIMS

1. An anti-CSF1R antibody or an antigen-binding portion thereof, wherein the
5 antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein
 - (a) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32) and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence
10 comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38) and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);
 - (b) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises
15 LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60) and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);
 - (c) the VH region amino acid sequence comprises HCDR1 of GYTFSSAYMI (SEQ ID NO:57), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32) and HCDR3 of EGPYFSNLYVMDY (HCDR3; SEQ ID NO: 33); and the VL region amino acid sequence
20 comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO:37), LCDR2 of AASDRAT (SEQ ID NO:60) and LCDR3 of QLSNEDLLT (SEQ ID NO:39); or
 - (d) the VH region amino acid sequence comprises HCDR1 of GYTFTSYMYI (SEQ ID NO:41), HCDR2 of MGDINPYNGGTTYAQKFQG (SEQ ID NO:42) and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO:33); and the VL region amino acid sequence comprises
25 LCDR1 of RASQSVSYEGENYLA (SEQ ID NO:40), LCDR2 of AASNLAT (SEQ ID NO:43) and LCDR3 of QLSNEDLLT (SEQ ID NO:39).
2. The antibody or antigen-binding portion of claim 1, wherein
 - (a) the VH region amino acid sequence comprises SEQ ID NO:352 and the VL
30 region amino acid sequence comprises SEQ ID NO:351;
 - (b) the VH region amino acid sequence comprises SEQ ID NO:354 and the VL region amino acid sequence comprises SEQ ID NO:353;
 - (c) the VH region amino acid sequence comprises SEQ ID NO:356 and the VL region amino acid sequence comprises SEQ ID NO:355; or
 - (d) the VH region amino acid sequence comprises SEQ ID NO:358 and the VL
35 region amino acid sequence comprises SEQ ID NO:357.

3. An anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region comprising HCDR1, HCDR2, and HCDR3 and a light chain variable (VL) region comprising LCDR1, LCDR2, and LCDR3, wherein

5 (a) the HCDR1 comprises the amino acid sequence G-X₁-T-F-X₂-X₃-X₄-X₅-X₆-X₇, wherein X₁ is Y or G, X₂ is T or S, X₃ is D or S, X₄ is N, A, S, H, or Y, X₅ is Y or A, X₆ is M or I, and X₇ is I or S (SEQ ID NO: 25);

(b) the HCDR2 comprises the amino acid sequence X₁-G-X₂-I-X₃-P-X₄-X₅-G-X₆-X₇-X₈-X₉-X₁₀-Q-K-F-X₁₁-G, wherein X₁ is M or a conservative substitution of M, X₂ is D or G, 10 X₃ is N or I, X₄ is Y or I, X₅ is N or F, X₆ is G or T, X₇ is T or A, X₈ is T or N, X₉ is F or Y, X₁₀ is N or A, and X₁₁ is Q or K (SEQ ID NO: 26);

(c) the HCDR3 comprises the amino acid sequence X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-V-M-D-Y, wherein X₁ is E or a conservative substitution of E, X₂ is D, G, H, S, T, P, V, N, I, or Y, X₃ is P, T, N, E, L, A, D, or S, X₄ is Y, K, R, M, or P, X₅ is F, D, S, T, E, W, M, Y, L, Q, K, 15 G, A, or I, X₆ is S, E, G, or R, X₇ is N, E, Q, G, H, or M, X₈ is L, H, S, or Y, and X₉ is Y or W (SEQ ID NO: 27);

(d) the LCDR1 comprises the amino acid sequence R-A-S-Q-S-V-X₁-Y-X₂-G-X₃-N-Y-L-X₄, wherein X₁ is S, D, or E, X₂ is D, E, or Q, X₃ is D or E, and X₄ is N or A (SEQ ID NO: 28);

20 (e) the LCDR2 comprises the amino acid sequence X₁-A-S-X₂-X₃-X₄-T, wherein X₁ is A or D, X₂ is D or N, X₃ is L or R, and X₄ is E or A (SEQ ID NO: 29); and

(f) the LCDR3 comprises the amino acid sequence X₁-X₂-S-X₃-X₄-X₅-L-X₆-T, wherein X₁ is Q, H, or N, X₂ is L or Q, X₃ is N or S, X₄ is E, Q, or N, X₅ is D or W, and X₆ is L or S (SEQ ID NO: 30).

25

4. An anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ 30 ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYQGGENYLA (SEQ ID NO: 34), LCDR2 of DASNRAT (SEQ ID NO: 35), and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(b) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ 35 ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO: 32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises

LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(c) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of
5 EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLA (SEQ ID NO:40), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(d) the VH region amino acid sequence comprises HCDR1 of GYTFTSYMYI (SEQ ID NO:41), HCDR2 of MGDINPYNGGTTAQKFQG (SEQ ID NO: 42), and HCDR3 of
10 EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLA (SEQ ID NO:40), LCDR2 of AASNLAT (SEQ ID NO:43), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(e) the VH region amino acid sequence comprises HCDR1 of GYTFTSYMYI (SEQ ID NO:41), HCDR2 of MGDINPYNGGANFAQKFQG (SEQ ID NO:44), and HCDR3 of
15 EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(f) the VH region amino acid sequence comprises HCDR1 of GYTFTSYMYI (SEQ ID NO:41), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of
20 EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(g) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of
25 EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYQGDNYLN (SEQ ID NO:45), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(h) the VH region amino acid sequence comprises HCDR1 of GYTFTSYMYI (SEQ ID NO:46), HCDR2 of MGDINPYNGGATYAQKFQG (SEQ ID NO:47), and HCDR3 of
30 EPPYFSNLYVMDY (SEQ ID NO:48); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYEGDNYLN (SEQ ID NO:49), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(i) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGATYNQKFQG (SEQ ID NO:51), and HCDR3 of
35 EPPYFSNLYVMDY (SEQ ID NO:48); and the VL region amino acid sequence comprises

LCDR1 of RASQSVEYEGENYLN (SEQ ID NO:52), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(j) the VH region amino acid sequence comprises HCDR1 of GYTFSSYYMI (SEQ ID NO: 31), HCDR2 of MGDINPYNGGTTTAAQKFQG (SEQ ID NO: 42), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYQGENYLN (SEQ ID NO:53), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNQDLLT (SEQ ID NO: 36);

(k) the VH region amino acid sequence comprises HCDR1 of GYTFTSNYII (SEQ ID NO:54), HCDR2 of MGDINPYNGGTNYAQKFQG (SEQ ID NO:55), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVEYQGENYLN (SEQ ID NO:53), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSSDLLT (SEQ ID NO:56);

(l) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(m) the VH region amino acid sequence comprises HCDR1 of GYTFSSAYMI (SEQ ID NO:57), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(n) the VH region amino acid sequence comprises HCDR1 of GYTFSSSYMI (SEQ ID NO:58), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(o) the VH region amino acid sequence comprises HCDR1 of GYTFSSHYMI (SEQ ID NO:59), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASNRAT (SEQ ID NO:38), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(p) the VH region amino acid sequence comprises HCDR1 of GYTFSSNYMI (SEQ ID NO:50), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises

LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(q) the VH region amino acid sequence comprises HCDR1 of GYTFSSAYMI (SEQ ID NO:57), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39);

(r) the VH region amino acid sequence comprises HCDR1 of GYTFSSSYMI (SEQ ID NO:58), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39); or

(s) the VH region amino acid sequence comprises HCDR1 of GYTFSSHYMI (SEQ ID NO:59), HCDR2 of MGDINPYNGGANYAQKFQG (SEQ ID NO:32), and HCDR3 of EGPYFSNLYVMDY (SEQ ID NO: 33); and the VL region amino acid sequence comprises LCDR1 of RASQSVSYEGENYLN (SEQ ID NO: 37), LCDR2 of AASDRAT (SEQ ID NO:60), and LCDR3 of QLSNEDLLT (SEQ ID NO: 39).

5. An anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH region amino acid sequence comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; or SEQ ID NO:8; and the VL region amino acid sequence comprises SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11 or SEQ ID NO:12.

25

6. The antibody or antigen-binding portion of claim 5, wherein

(a) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:9;

(b) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:10;

(c) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:11;

(d) the VH region amino acid sequence comprises SEQ ID NO:1, and the VL region amino acid sequence comprises SEQ ID NO:12;

(e) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:9;

- (f) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:10;
- (g) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:11;
- 5 (h) the VH region amino acid sequence comprises SEQ ID NO:2, and the VL region amino acid sequence comprises SEQ ID NO:12;
- (i) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (j) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:10;
- 10 (k) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:11;
- (l) the VH region amino acid sequence comprises SEQ ID NO:3, and the VL region amino acid sequence comprises SEQ ID NO:12;
- 15 (m) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (n) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:10;
- (o) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:11;
- 20 (p) the VH region amino acid sequence comprises SEQ ID NO:4, and the VL region amino acid sequence comprises SEQ ID NO:12;
- (q) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:9;
- 25 (r) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:10;
- (s) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:11;
- (t) the VH region amino acid sequence comprises SEQ ID NO:5, and the VL region amino acid sequence comprises SEQ ID NO:12;
- 30 (u) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (v) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL region amino acid sequence comprises SEQ ID NO:10;
- 35 (w) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL region amino acid sequence comprises SEQ ID NO:11;

- (x) the VH region amino acid sequence comprises SEQ ID NO:6, and the VL region amino acid sequence comprises SEQ ID NO:12;
- (y) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL region amino acid sequence comprises SEQ ID NO:9;
- 5 (z) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL region amino acid sequence comprises SEQ ID NO:10;
- (aa) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL region amino acid sequence comprises SEQ ID NO:11;
- (bb) the VH region amino acid sequence comprises SEQ ID NO:7, and the VL
- 10 region amino acid sequence comprises SEQ ID NO:12;
- (cc) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:9;
- (dd) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:10;
- 15 (ee) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:11; or
- (ff) the VH region amino acid sequence comprises SEQ ID NO:8, and the VL region amino acid sequence comprises SEQ ID NO:12.
- 20 7. An anti-CSF1R antibody or an antigen-binding portion thereof, wherein the antibody or antigen-binding portion cross-competes for binding to CSF1R with the antibody or antigen-binding portion of any one of claims 1-6; and
- (a) comprises fully germline human framework amino acid sequences;
- (b) comprises the IGHV1-69*01 allele germline framework regions in the VH
- 25 domain;
- (c) does not contain an isomerisation risk motif in the LCDR1 sequence;
- (d) binds specifically to human CSF1R, cynomolgus CSF1R, and rhesus CSF1R; and/or
- (e) binds to human CSF1R with a KD that is within 3-fold of said antibody's or
- 30 antigen-binding portion's KD for binding to cynomolgus CSF1R;
- (f) does not comprise a human T cell epitope sequence in the Framework 3/LCDR3 region of the VL domain; and/or
- (g) does not comprise a human T cell epitope sequence in the HCDR-2/Framework 3 region of the VH domain; and/or
- 35 (h) does not comprise a human T cell epitope sequence in the HCDR1/Framework 2 region of the VH domain; and/or

- (i) does not comprise a human T cell epitope sequence in the HCDR3/Framework 4 region of the VH domain; and/or
- (j) does not comprise a human T cell epitope sequence in the Framework 2/LCDR2 region of the VL domain; and/or
- 5 (k) exhibits minimal or no binding to insulin and/or double-stranded DNA; and/or
- (l) exhibits reduced susceptibility to oxidative damage compared to antibody hu0301, when in IgG4(S228P) antibody format; and/or
- (m) exhibits improved thermal stability in the Fab domain compared to antibody hu0301, when in IgG4(S228P) antibody format; and/or
- 10 (n) exhibits reduced hydrophobic species heterogeneity compared to antibody hu0301, when in IgG4(S228P) antibody format.
8. The antibody or antigen-binding portion of any one of claims 1-6, wherein the antibody is human, humanized or chimeric.
- 15 9. The antibody or antigen-binding portion of any one of claims 1-8, wherein the VH region, the VL region, or both the VH and the VL region comprise one or more human framework region amino acid sequences.
- 20 10. The antibody or antigen-binding portion of any one of claims 1-9, wherein the VH region, the VL region, or both the VH and the VL region comprise a human variable region framework scaffold amino acid sequence into which the CDRs have been inserted.
- 25 11. The antibody or antigen-binding portion of any one of claims 1, 3, 4 and 7 wherein the VH region comprises an IGHV1-69*01 human germline scaffold amino acid sequence into which the HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted.
- 30 12. The antibody or antigen-binding portion of any one of claims 1, 3, 4, 7 and 11, wherein the VL region comprises an IGKV3-11 human germline scaffold amino acid sequence into which the LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted.
- 35 13. The antibody or antigen-binding portion of any one of claims 1-12, wherein the antibody comprises an immunoglobulin constant region.

14. The antibody or antigen-binding portion of claim 13, wherein the immunoglobulin constant region is IgG, IgE, IgM, IgD, IgA or IgY.
15. The antibody or antigen-binding portion of claim 14, wherein the immunoglobulin constant region is IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2.
16. The antibody or antigen-binding portion of claim 13, wherein the immunoglobulin constant region is immunologically inert.
17. The antibody or antigen-binding portion of claim 13, wherein the immunoglobulin constant region is a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A.
18. The antibody or antigen-binding portion of claim 13, wherein the immunoglobulin constant region comprises any one of SEQ ID NOS:359-364.
19. The antigen-binding portion of any one of claims 1-18, wherein the antibody or antigen-binding portion is an Fab, an Fab', an F(ab')₂, an Fd, an Fv, an scFv, a single domain antibody (dAb), a maxibody, a minibody, an intrabody, a diabody, a triabody, a tetrabody, a v-NAR or a bis-scFv,
20. The antibody or antigen-binding portion of any one of claims 1-19, wherein the antibody is monoclonal.
21. The antibody or antigen-binding portion of any one of claims 1-20, wherein the antibody is a tetrameric antibody, a tetravalent antibody or a multispecific antibody.
22. The antibody or antigen-binding portion of any one of claims 1-21, wherein the antibody is a bispecific antibody that binds specifically to a first antigen and a second antigen, wherein the first antigen is CSF1R and the second antigen is not CSF1R.
23. The antibody or antigen-binding portion of any one of claims 1-22, wherein the antibody or antigen-binding portion binds specifically to (a) human CSF1R, (b) human

CSF1R and cynomolgus CSF1R, or (c) human CSF1R, cynomolgus CSF1R, and rhesus CSF1R.

24. An immunoconjugate comprising the antibody or antigen-binding portion of any one
5 of claims 1-23 linked to a therapeutic agent.

25. The immunoconjugate of claim 24, wherein the therapeutic agent is a cytotoxin, a
radioisotope, a chemotherapeutic agent, an immunomodulatory agent, an anti-angiogenic
agent, an antiproliferative agent, a pro-apoptotic agent, a cytostatic enzyme, a cytolytic
10 enzymes, a therapeutic nucleic acid, an anti-angiogenic agent, an anti-proliferative agent,
or a pro-apoptotic agent.

26. A pharmaceutical composition comprising the antibody or antigen-binding portion of
any one of claims 1-23 or the immunoconjugate of claim 24 or 25, and a pharmaceutically
15 acceptable carrier, diluent or excipient.

27. A nucleic acid molecule encoding
(a) the VH region amino acid sequence;
(b) the VL region amino acid sequence; or
20 (c) both the VH and the VL region amino acid sequences
of the antibody or antigen-binding portion of any one of claims 1-23.

28. An expression vector comprising the nucleic acid molecule of claim 27.

25 29. A recombinant host cell comprising the nucleic acid molecule of claim 27 or the
expression vector of claim 28.

30. A method of producing an anti-CSF1R antibody or an antigen-binding portion
thereof, the method comprising:
30 culturing a recombinant host cell comprising the expression vector of claim 28
under conditions whereby the nucleic acid molecule is expressed, thereby producing the
antibody or antigen-binding portion; and
isolating the antibody or antigen-binding portion from the host cell or culture.

35 31. A method for enhancing an immune response in a subject, comprising
administering to the subject a therapeutically effective amount of the antibody or antigen-

binding portion of any one of claims 1-23, the immunoconjugate of claim 24 or 25 or the pharmaceutical composition of claim 26.

32. A method of treating or preventing cancer, an infectious disease, or an immune
5 disease in a subject, comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding portion of any one of claims 1-23, the immunoconjugate of claim 24 or 25 or the pharmaceutical composition of claim 26.

33. The method of claim 32, wherein the cancer is pancreatic cancer, melanoma,
10 breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland
15 cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, or cancer of hematological tissues.

34. The method of claim 32, wherein the infectious disease is viral, bacterial, fungal, or
20 parasitic.

35. The method of claim 32, wherein the immune disease is pigmented villonodular synovitis.

36. The antibody or antigen-binding portion of any one of claims 1-23, the
25 immunoconjugate of claim 24 or 25 or the pharmaceutical composition of claim 26 for use in the treatment of cancer, an infectious disease, or an immune disease.

37. The antibody or antigen-binding portion, the immunoconjugate or the
30 pharmaceutical composition for use according to claim 36, wherein the cancer is pancreatic cancer, melanoma, breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer,
35 salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, or cancer of hematological tissues.

38. The antibody or antigen-binding portion, the immunoconjugate or the pharmaceutical composition for use according to claim 36, wherein the infectious disease is viral, bacterial, fungal, or parasitic.
- 5
39. The antibody or antigen-binding portion, the immunoconjugate or the pharmaceutical composition for use according to claim 36, wherein the immune disease is pigmented villonodular synovitis.
- 10
40. The antibody or antigen-binding portion of any one of claims 1-23, the immunoconjugate of claim 24 or 25 or the pharmaceutical composition of claim 26, for use as a medicament.



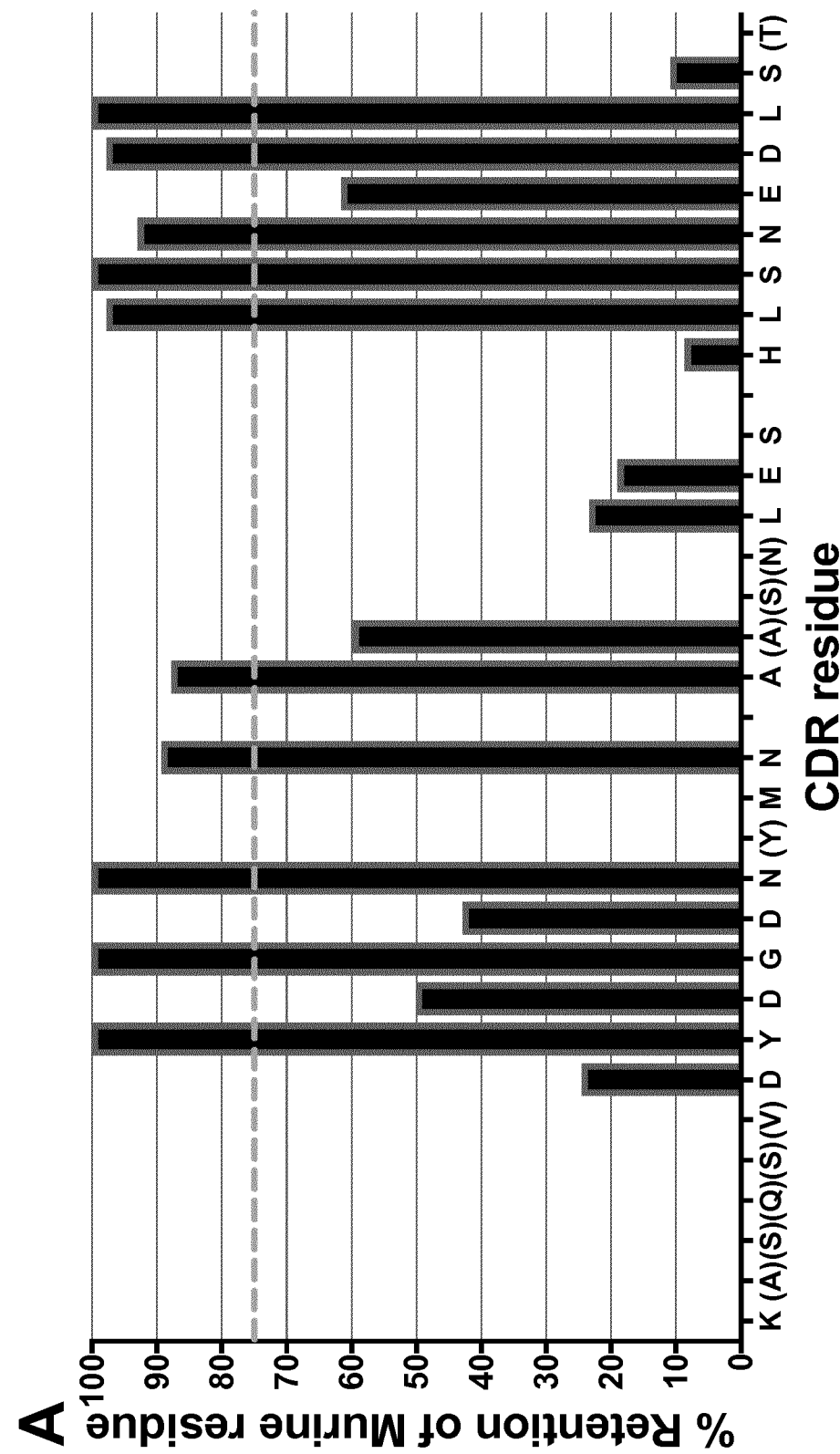


Fig. 2A

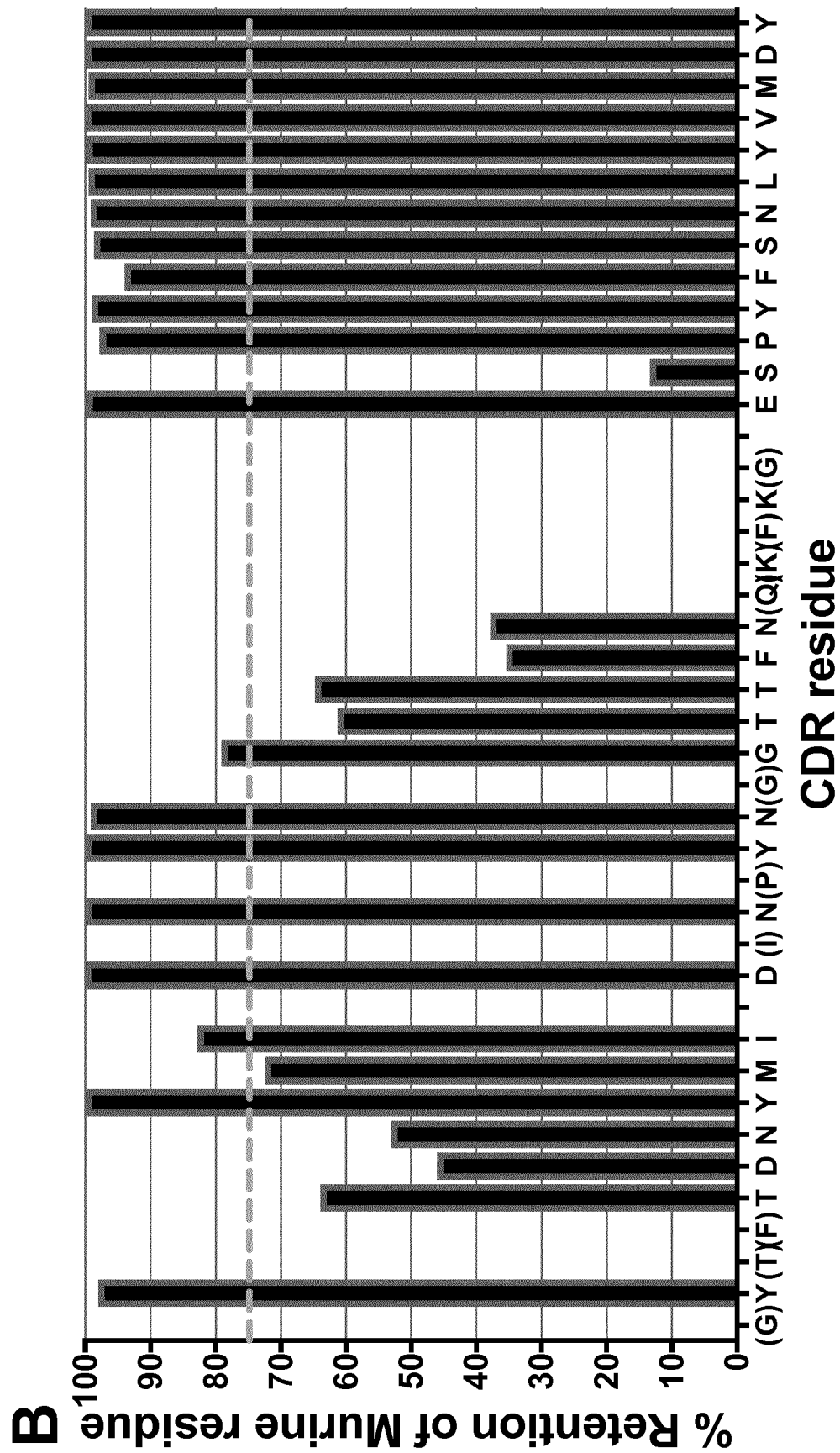


Fig. 2B

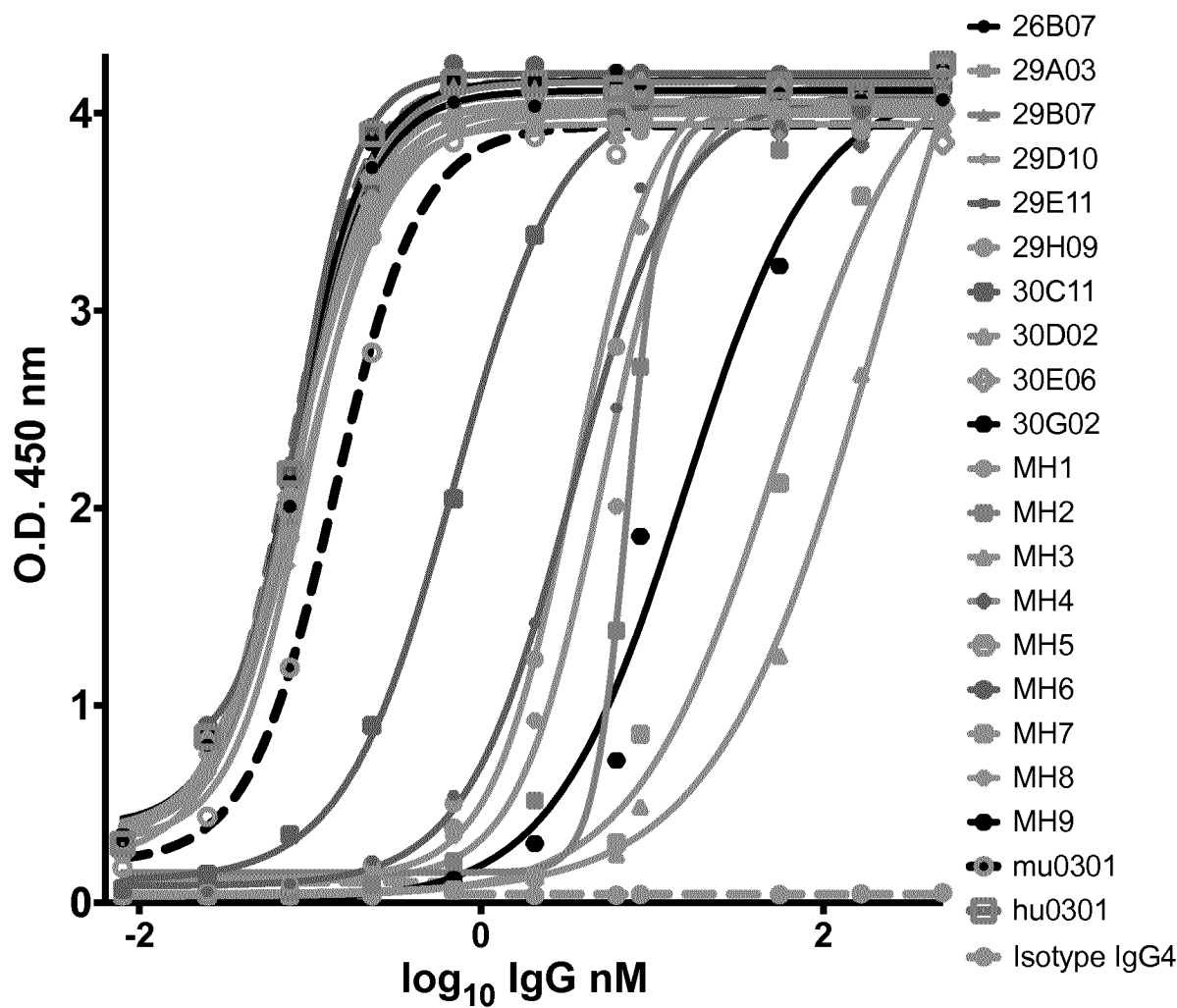


Fig. 3A

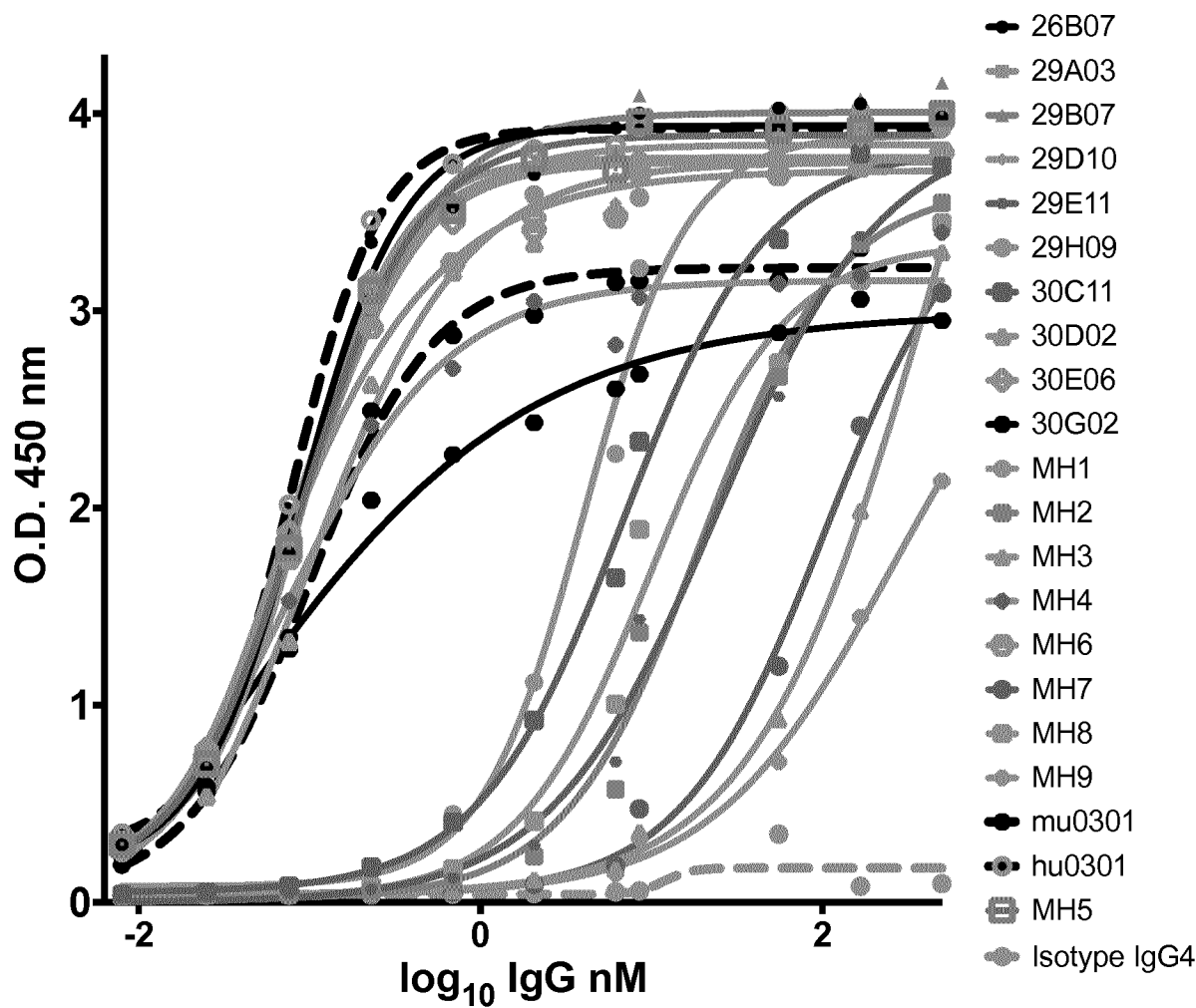


Fig. 3B

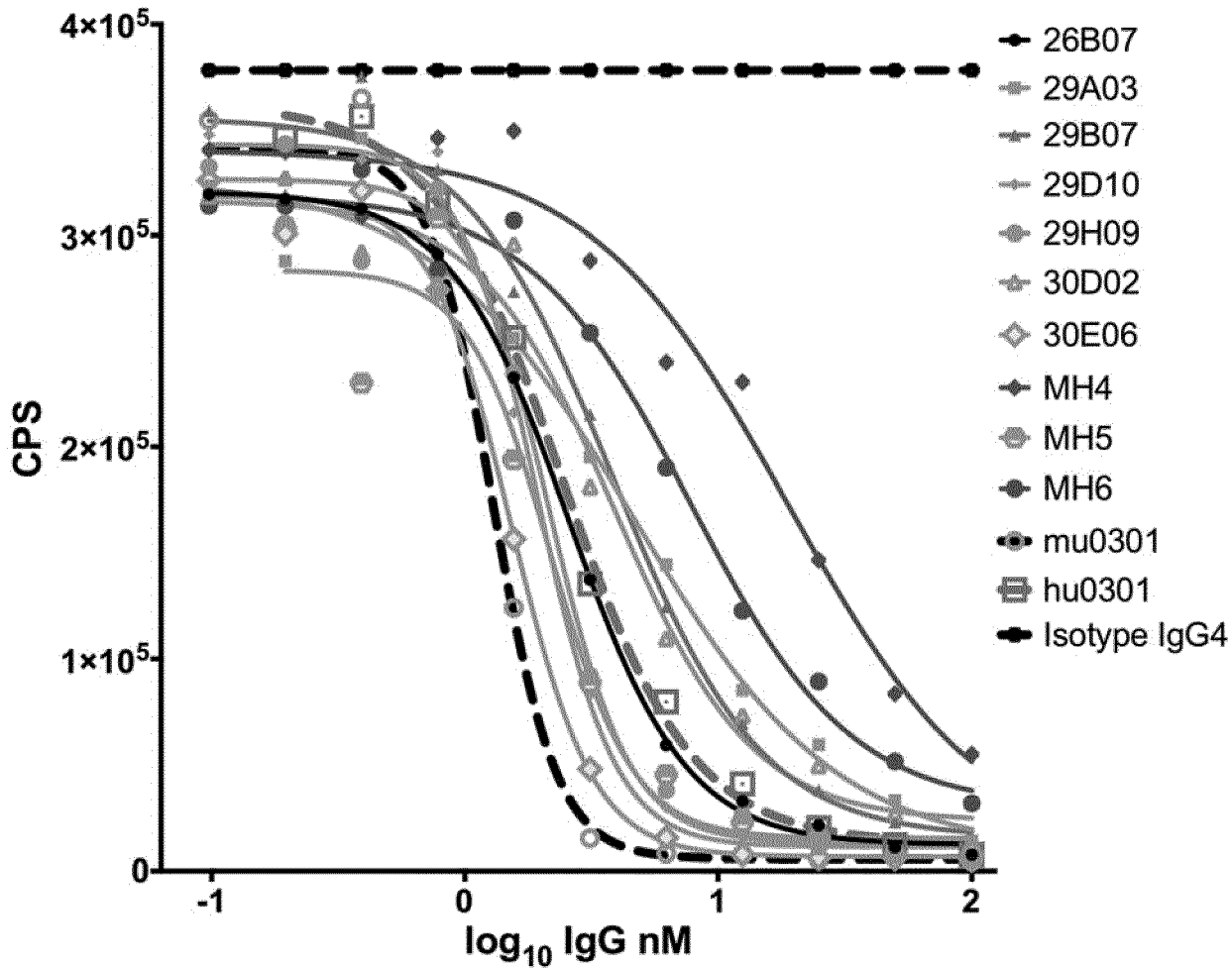


Fig 4

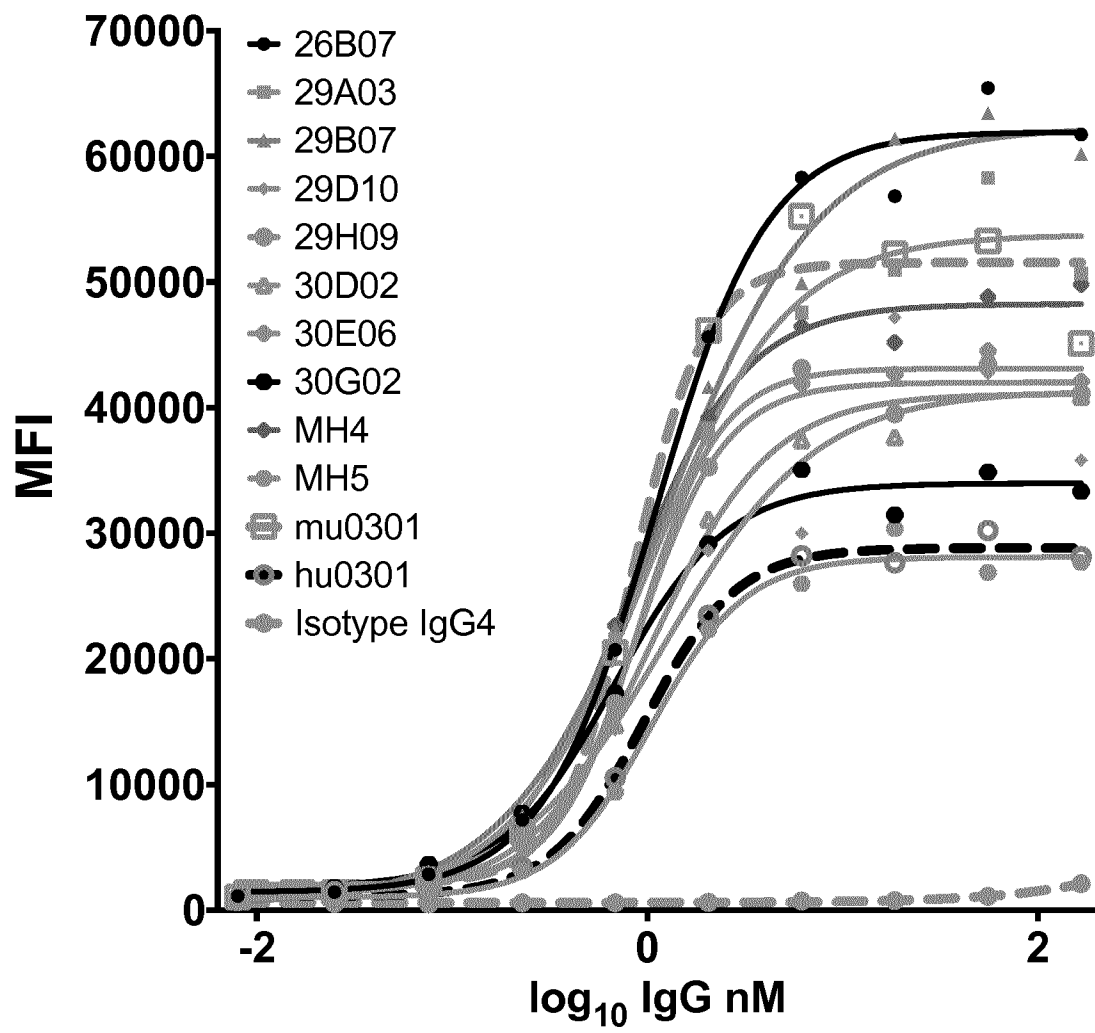


Fig. 5A

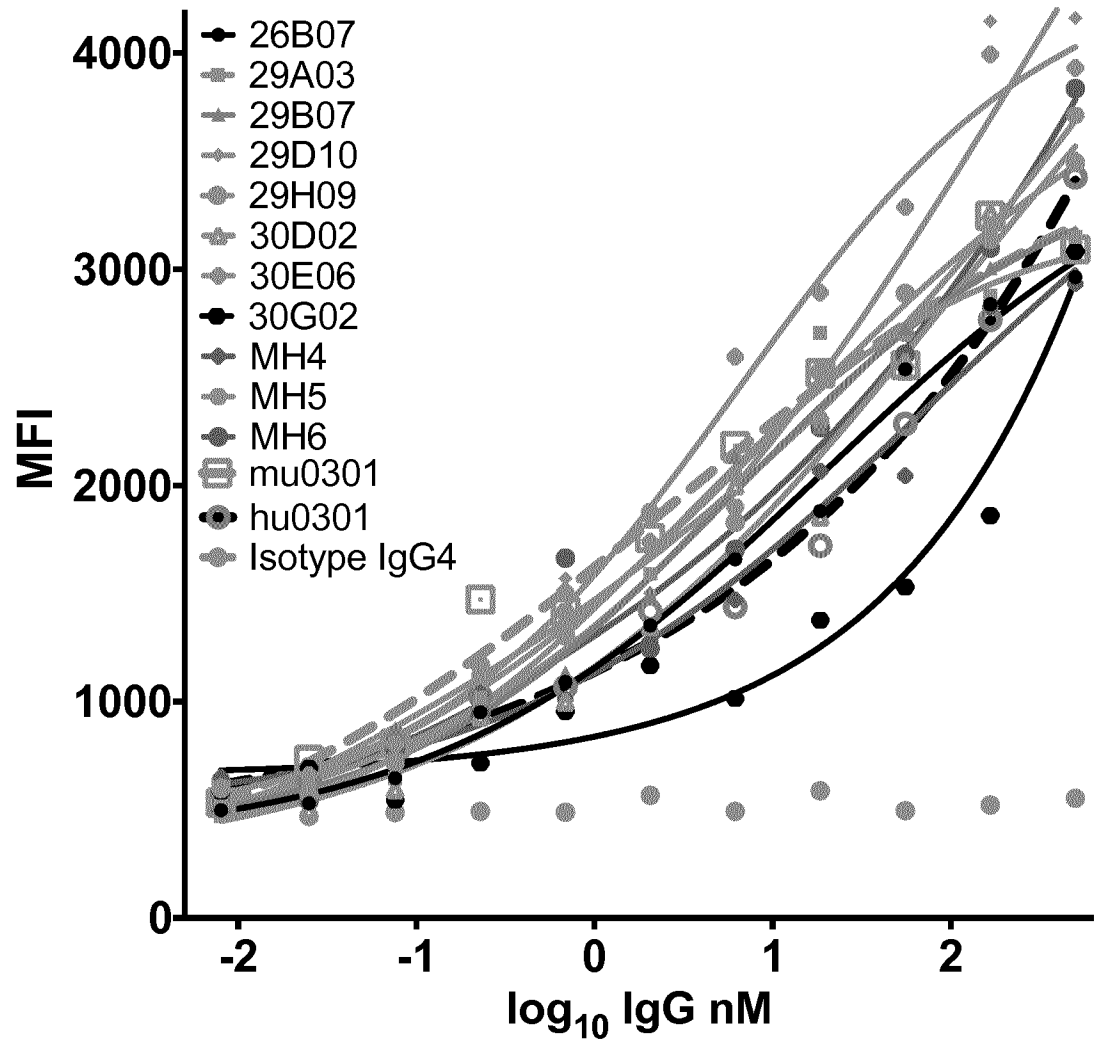


Fig. 5B

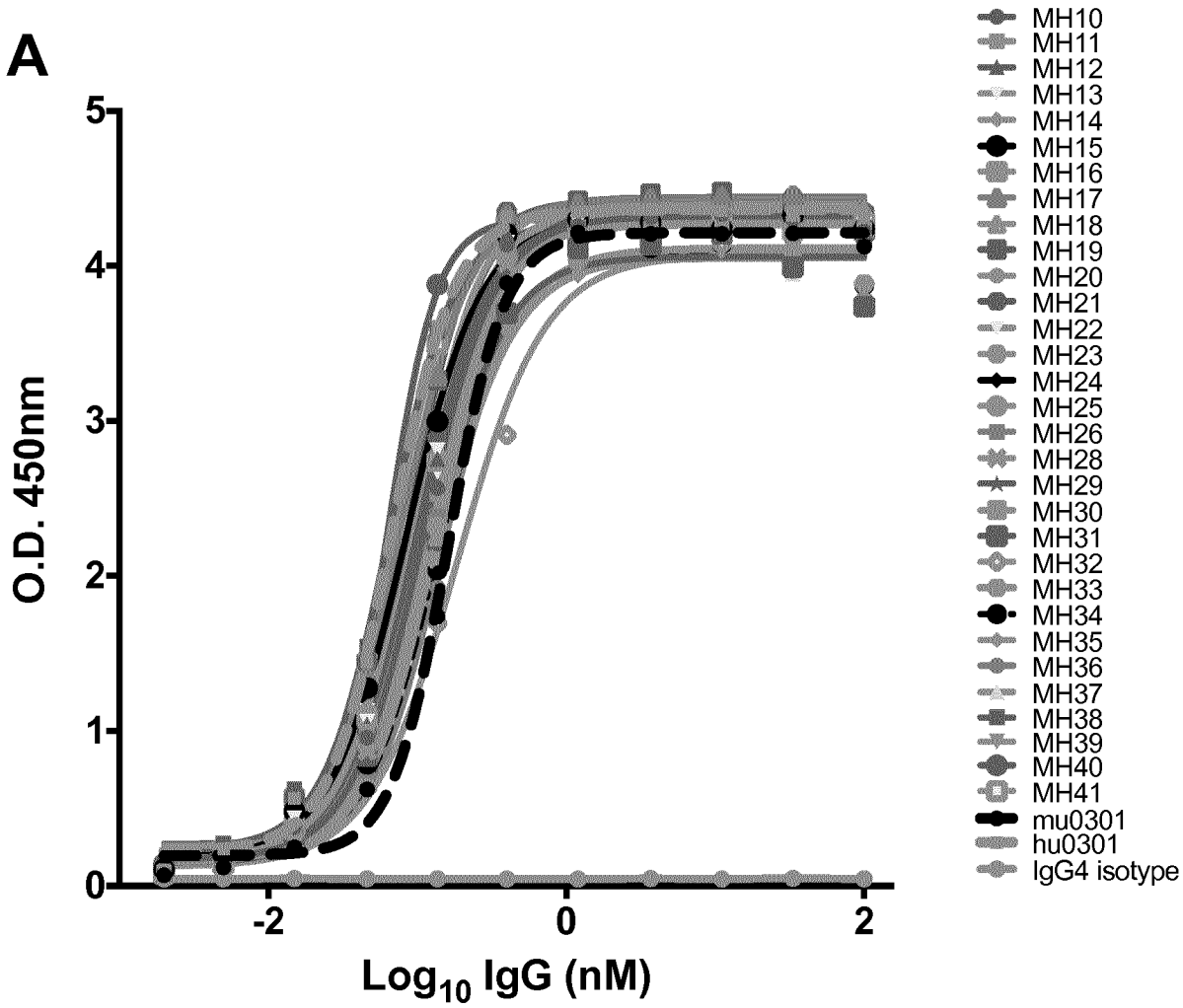


Fig. 6A

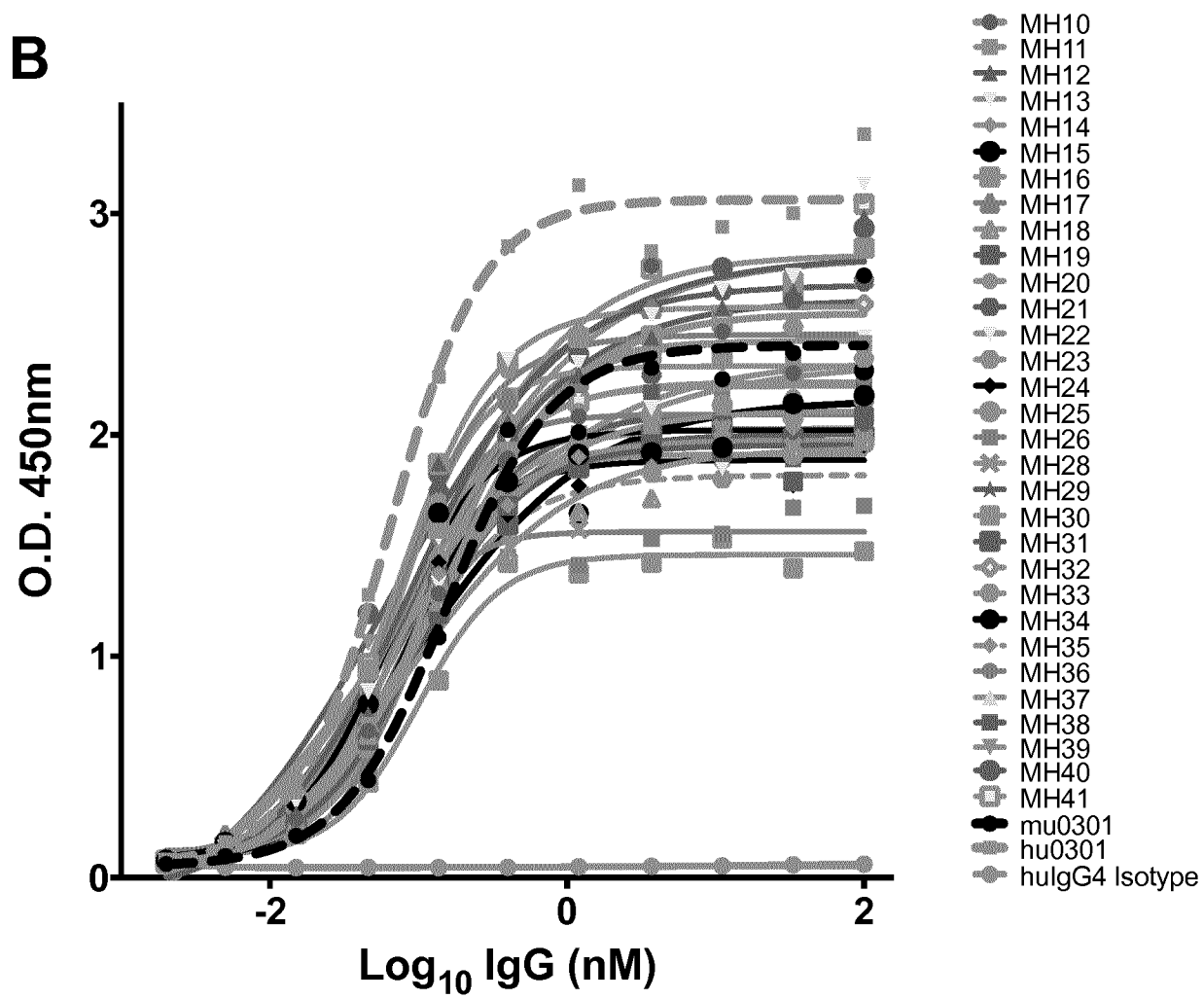


Fig. 6B

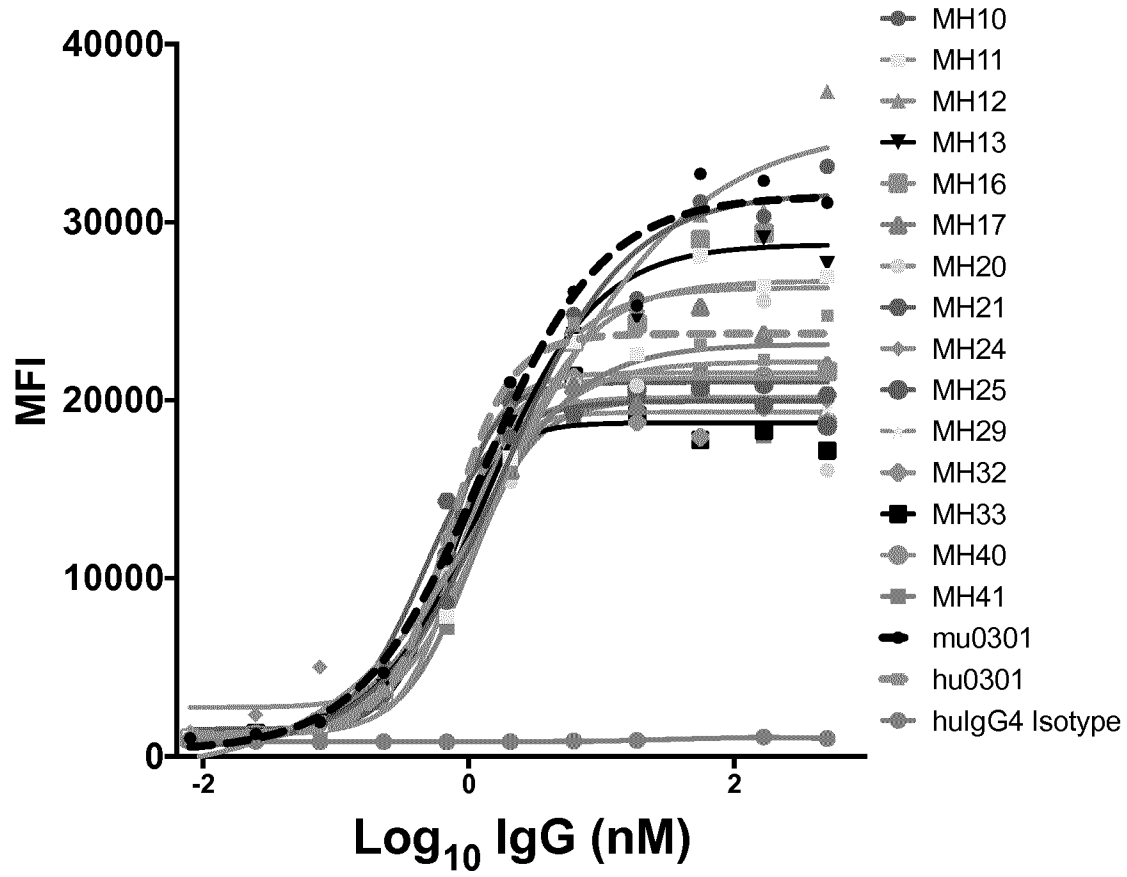


Fig. 7

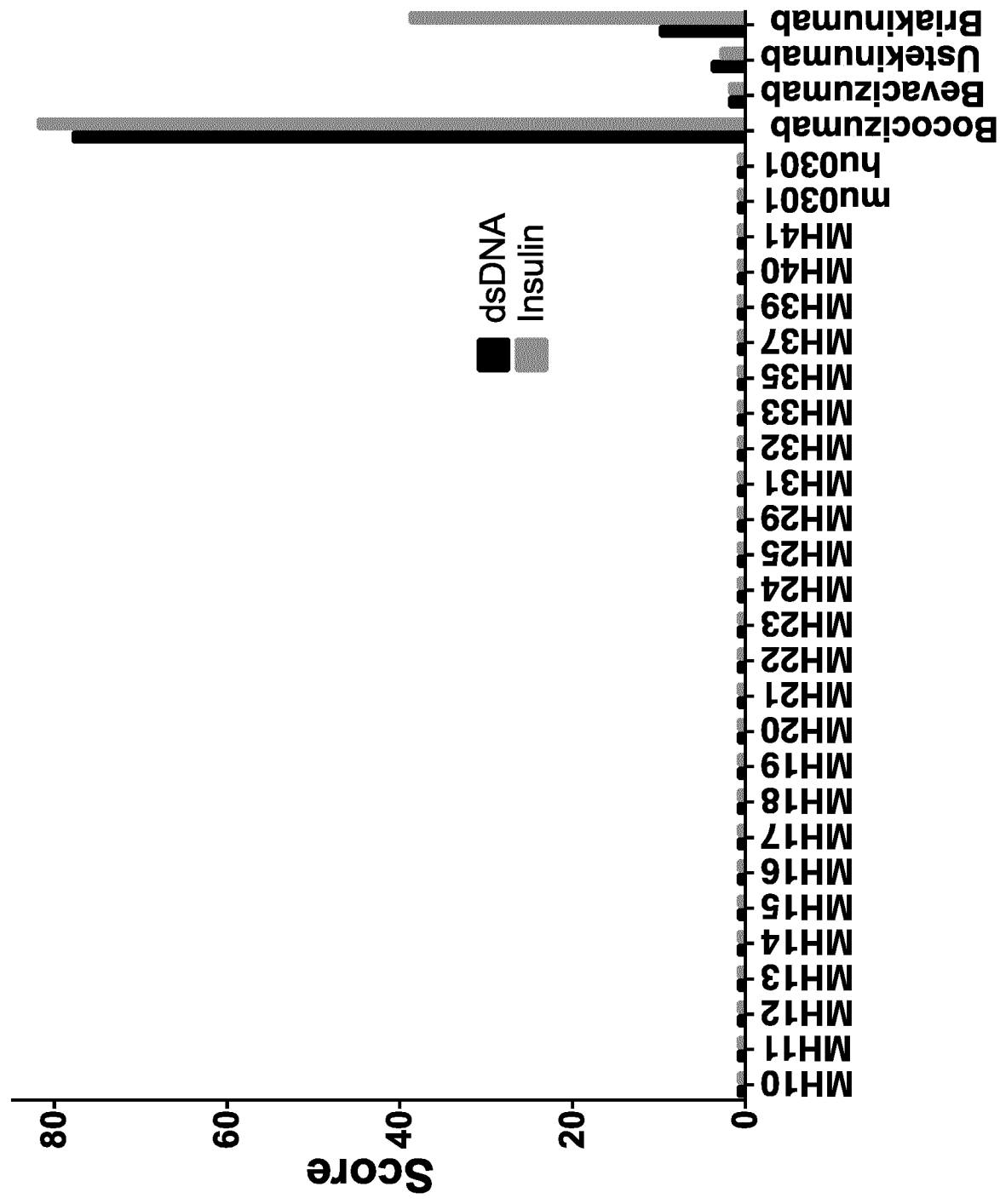


Fig. 8

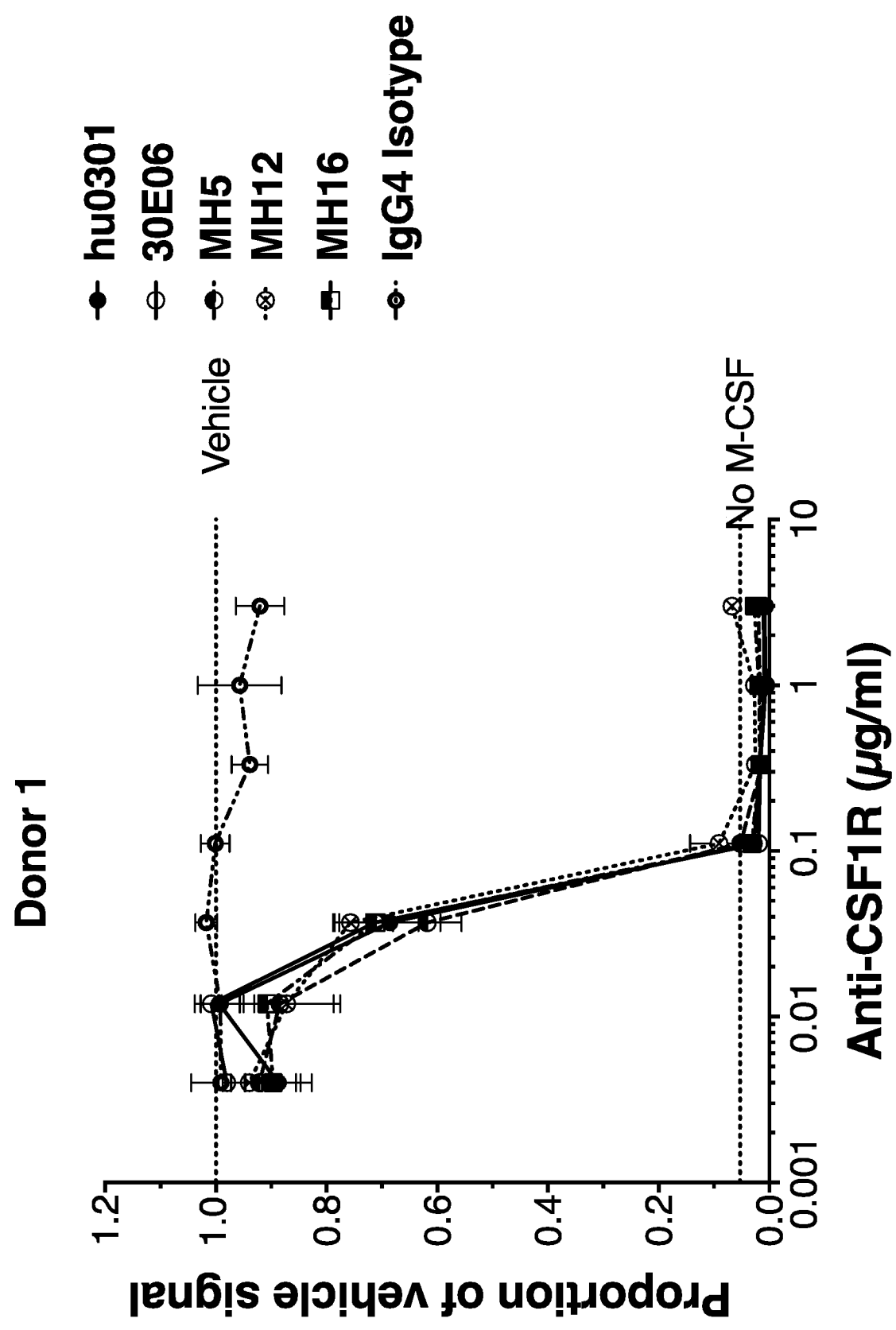


Fig. 9A

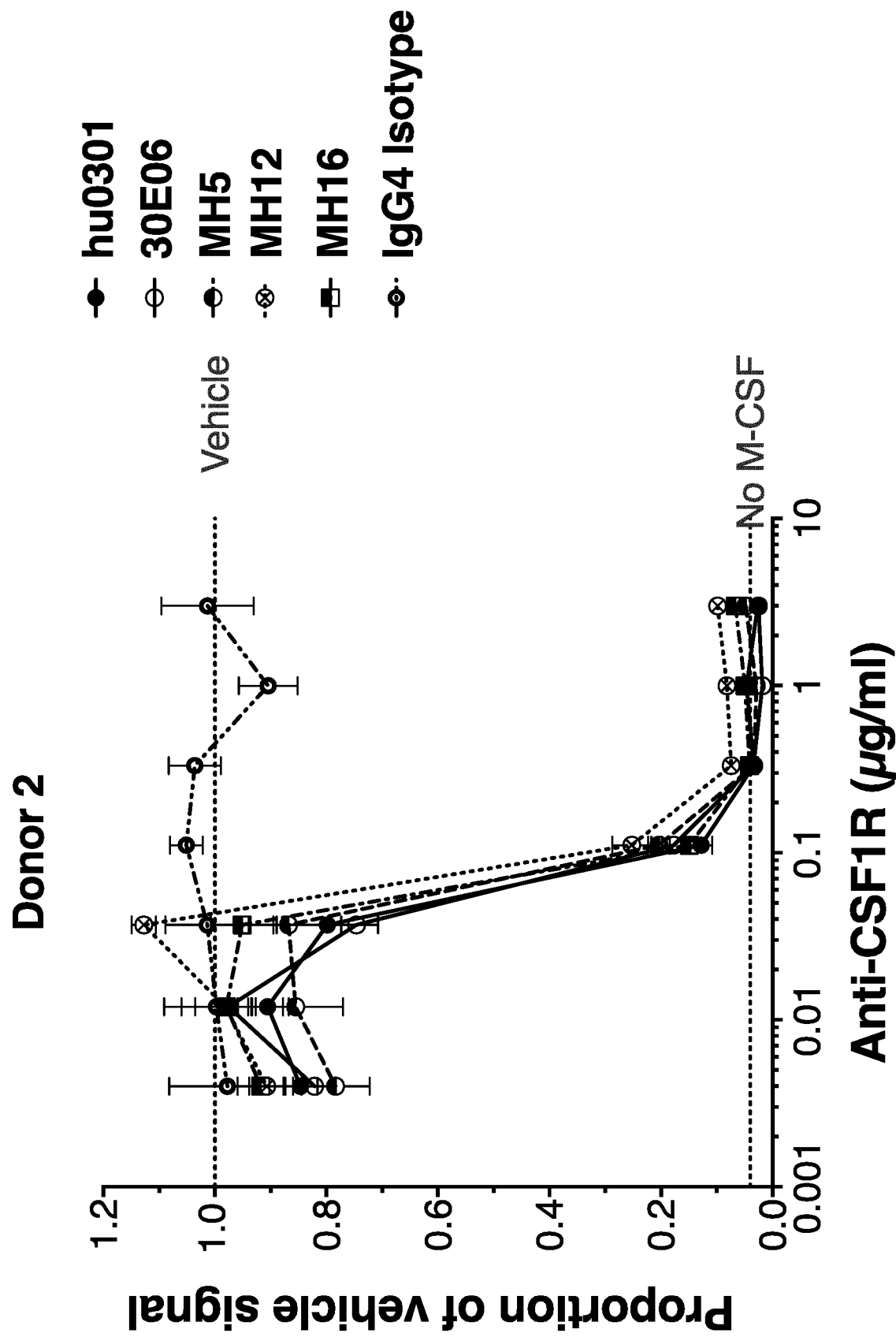


Fig. 9B

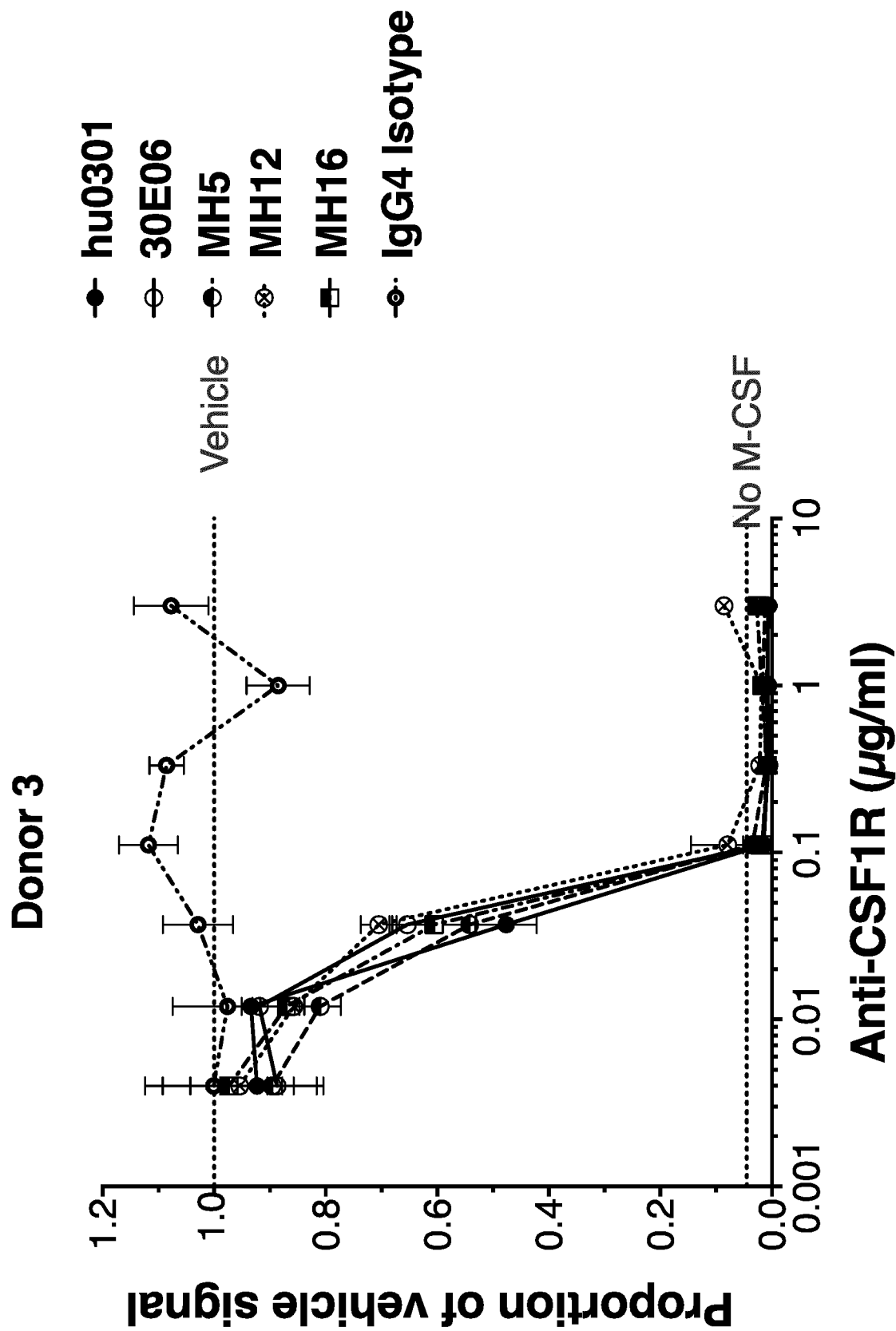


Fig. 9C

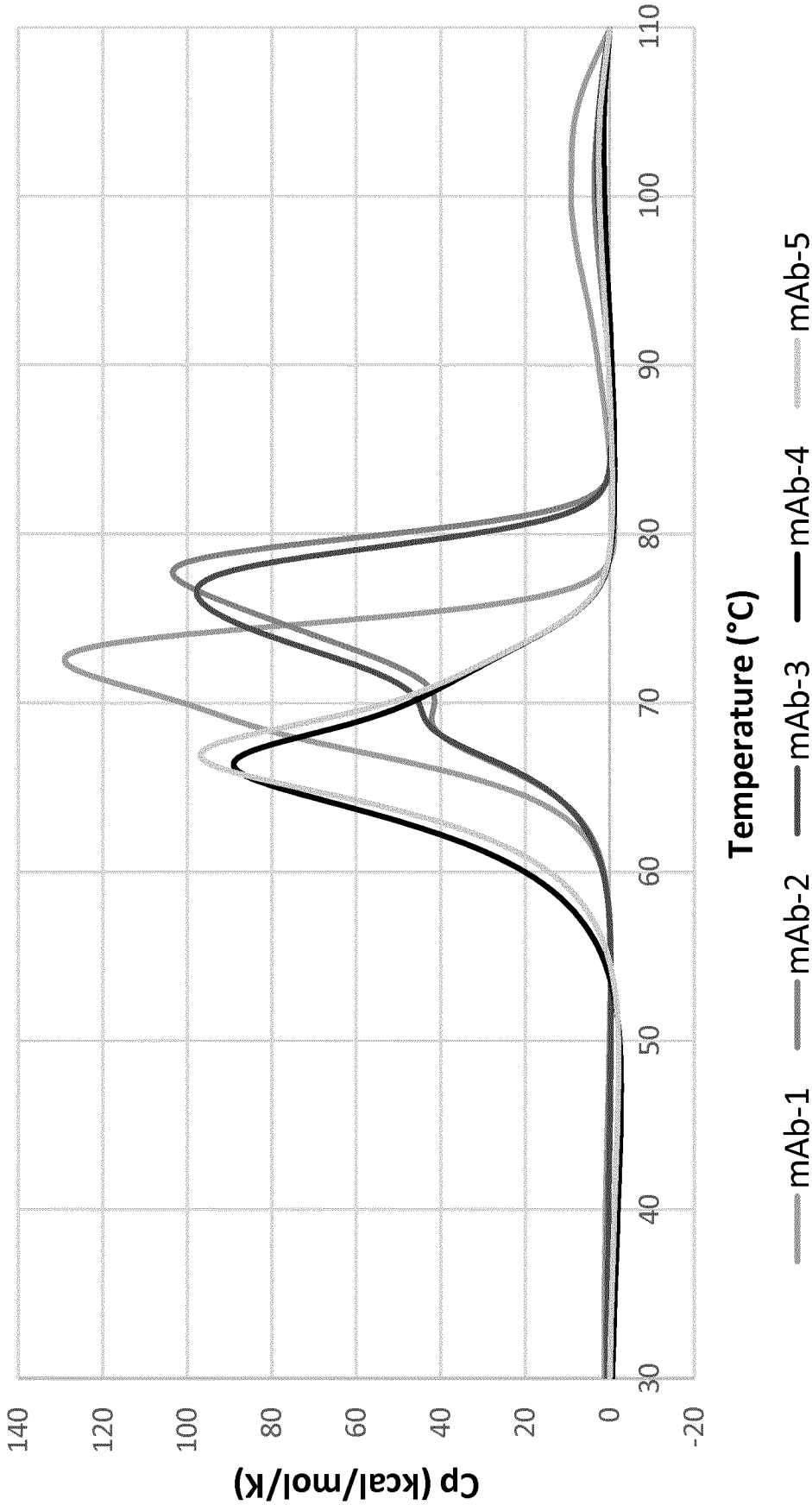


Fig. 10

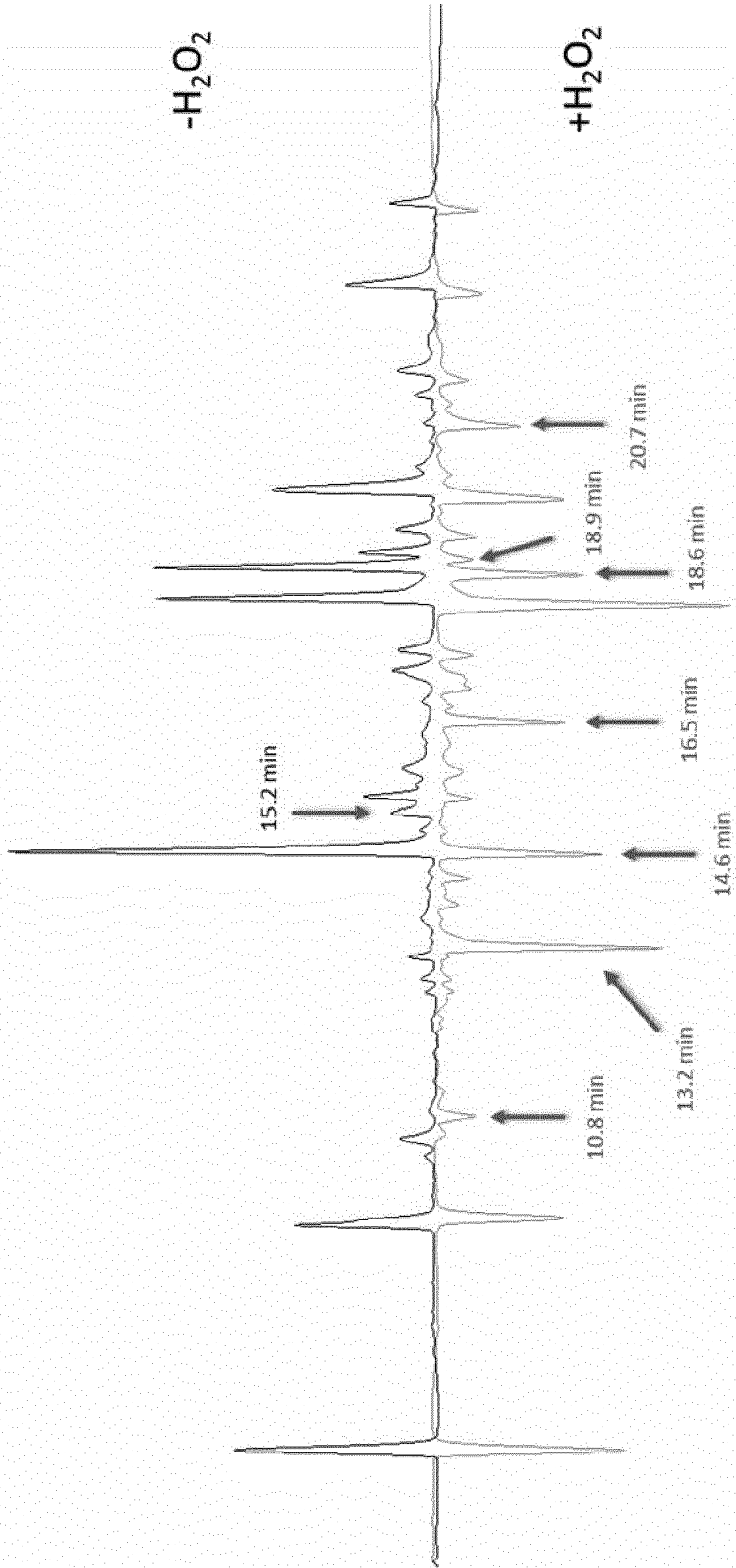


Fig. 11A

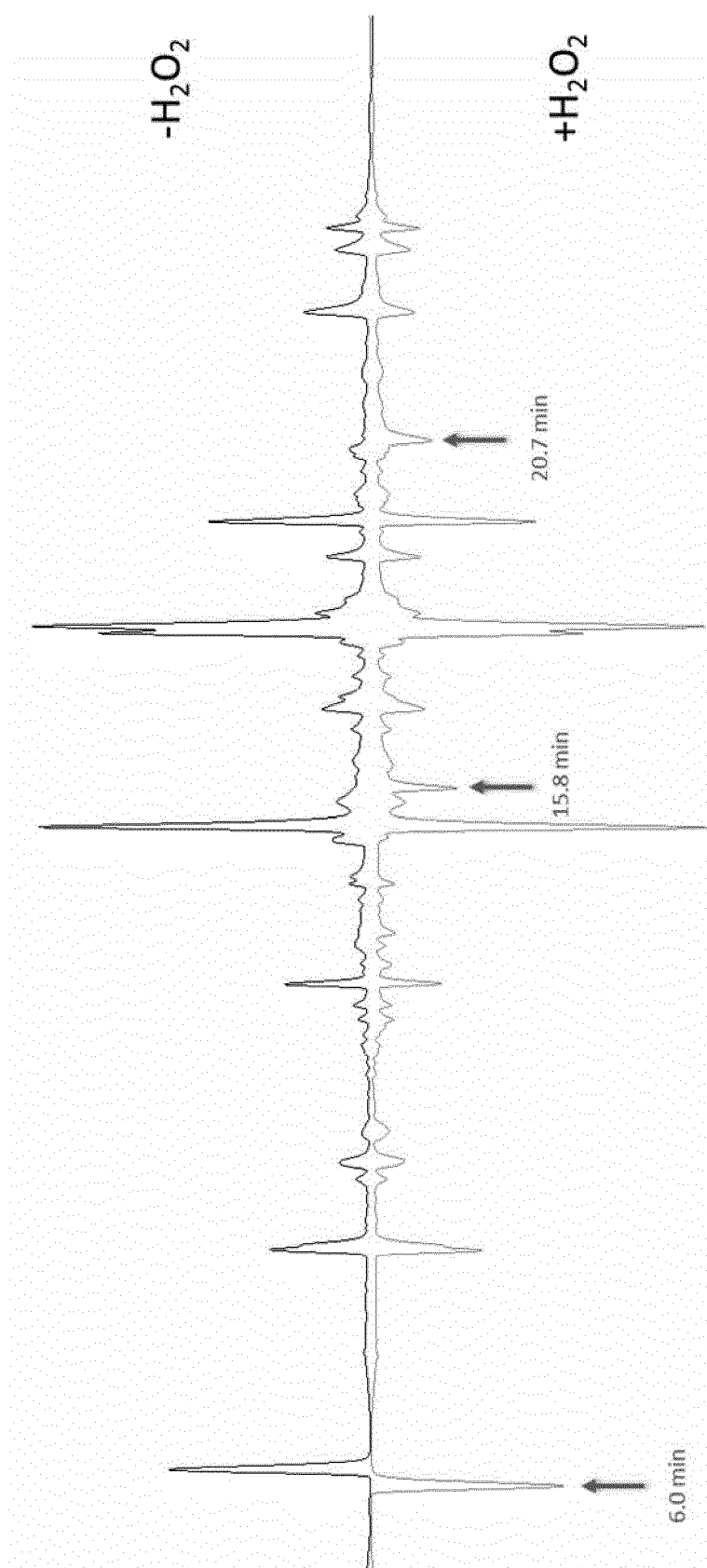


Fig. 11B

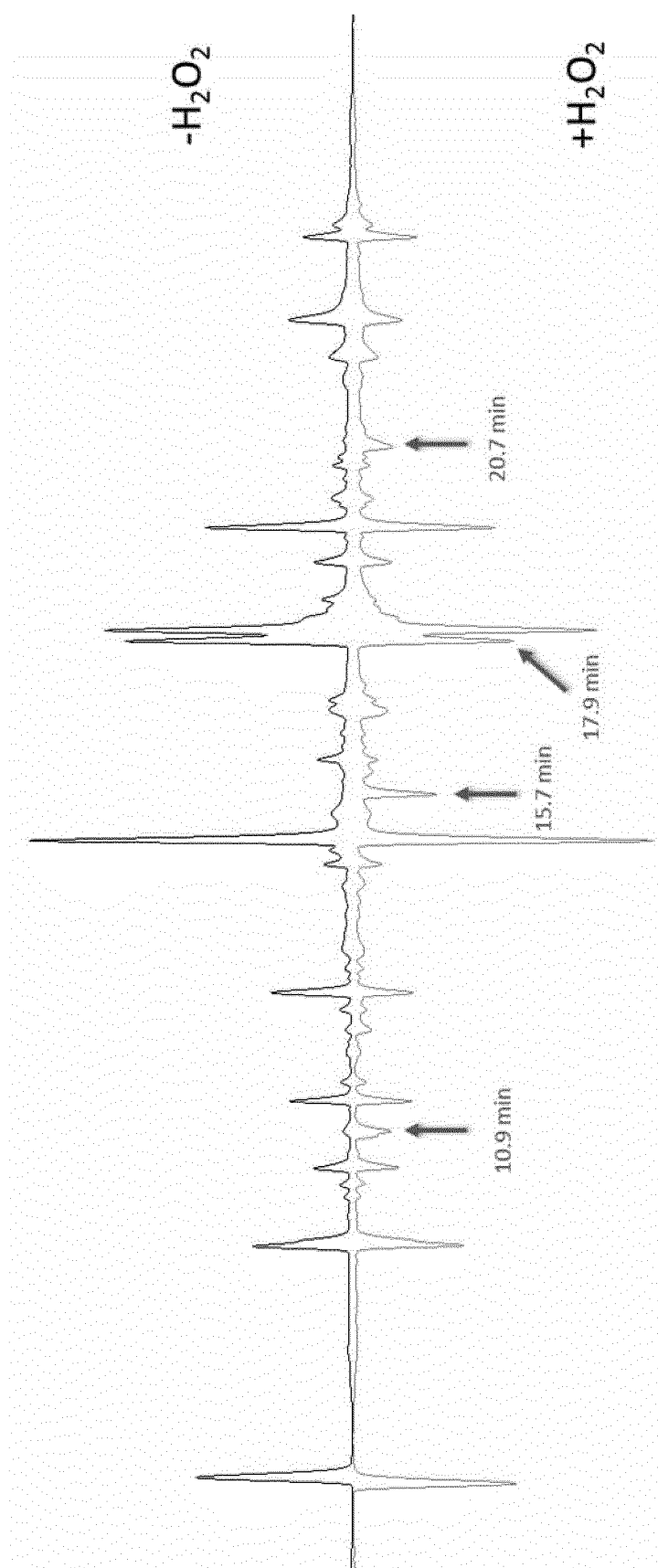


Fig. 11C

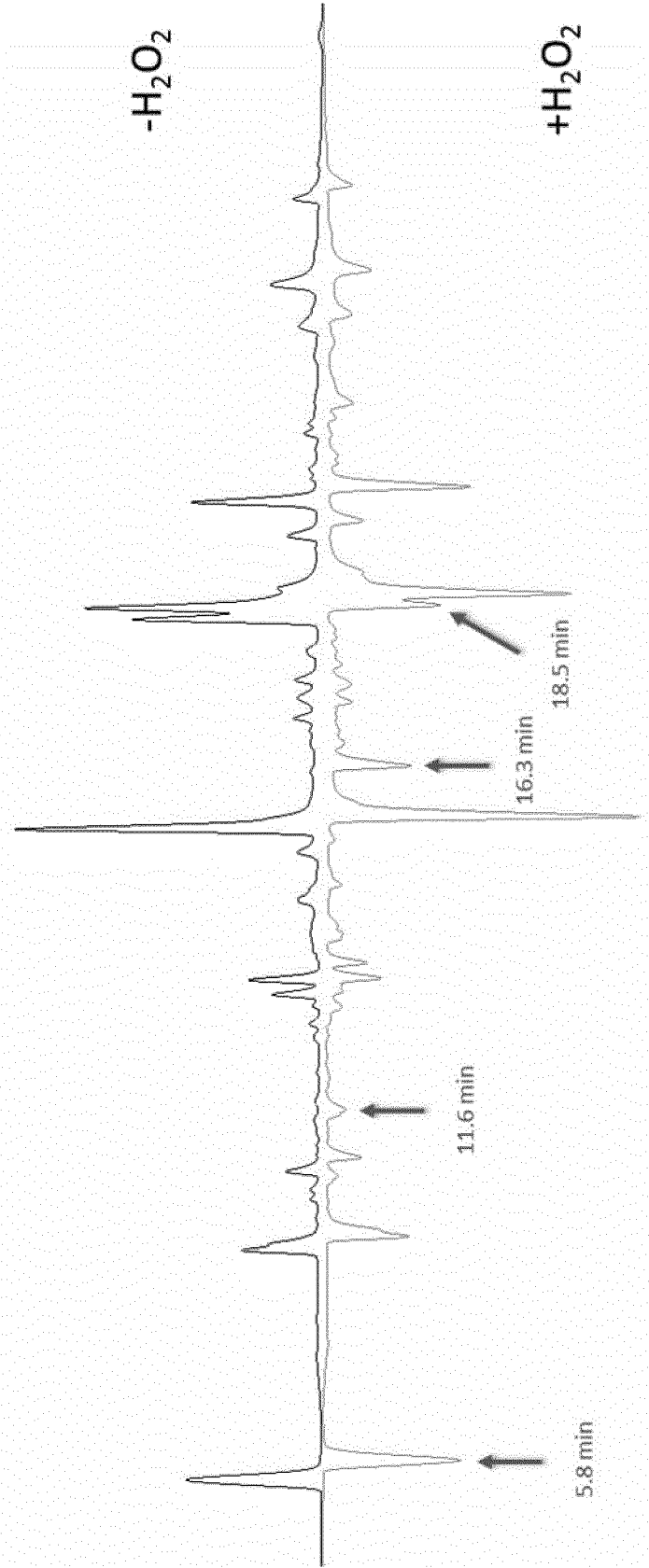


Fig. 11D

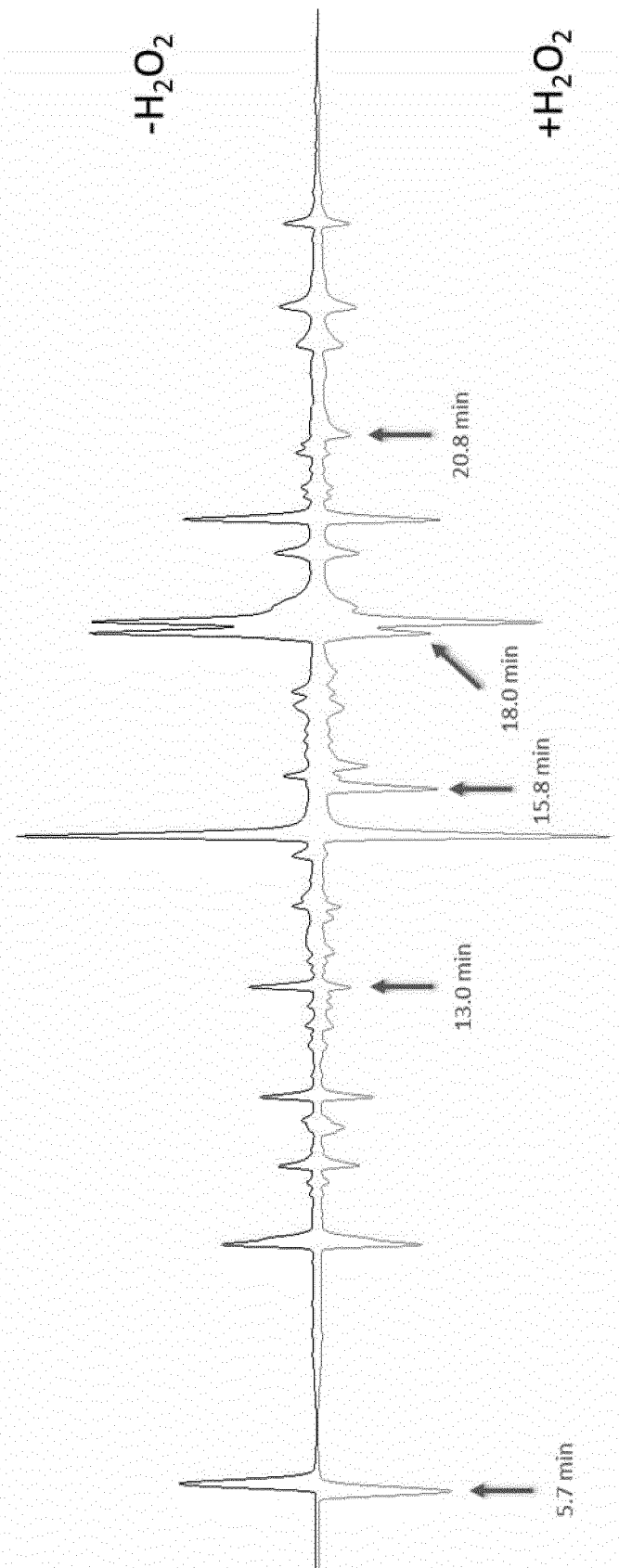


Fig. 11E

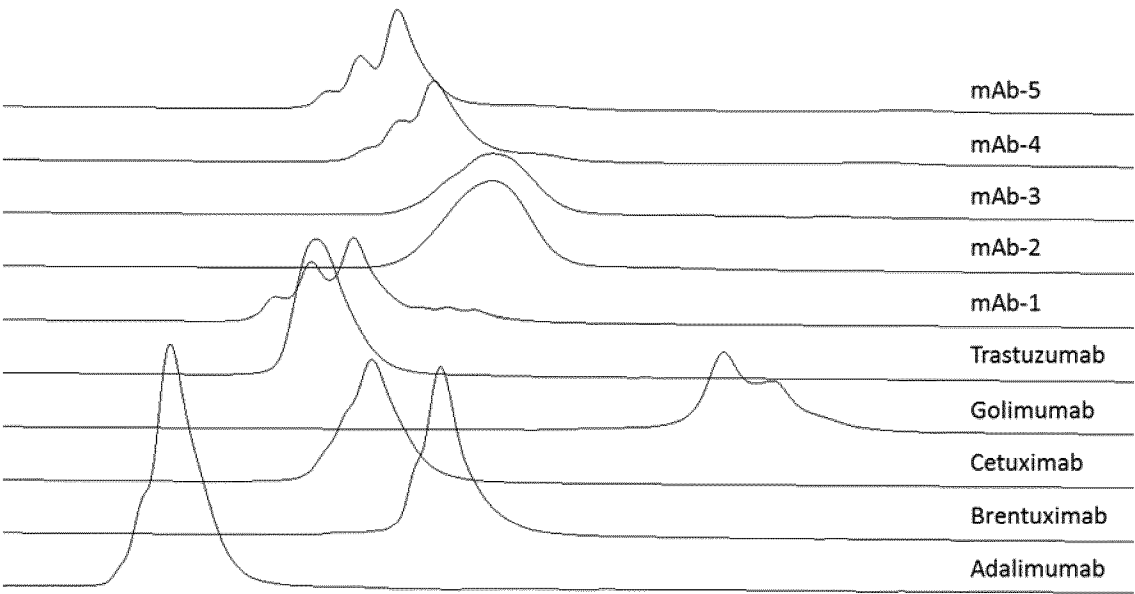


Fig. 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/055077

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
ADD.

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)
C07K

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)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO 2011/140249 A2 (FIVE PRIME THERAPEUTICS INC; WONG JUSTIN [US] ET AL.) 10 November 2011 (2011-11-10) cited in the application claims 1-83; examples 4,1,2,5-7; tables 4,5,2	1-40
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Further documents are listed in the continuation of Box C.



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Date of the actual completion of the international search

2 April 2019

Date of mailing of the international search report

11/04/2019

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Siaterli, Maria

INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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