



(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION
(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2018/04/19
(87) Date publication PCT/PCT Publication Date: 2018/10/25
(85) Entrée phase nationale/National Entry: 2019/10/02
(86) N° demande PCT/PCT Application No.: US 2018/028315
(87) N° publication PCT/PCT Publication No.: 2018/195283
(30) Priorité/Priority: 2017/04/19 (US62/487,061)

(51) Cl.Int./Int.Cl. *C07K 16/28* (2006.01),
A61K 39/00 (2006.01)
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(54) Titre : MOLECULES MULTISPECIFIQUES ET UTILISATIONS CORRESPONDANTES
(54) Title: MULTISPECIFIC MOLECULES AND USES THEREOF

(57) Abrégé/Abstract:

Multispecific molecules targeting tumor associated macrophages (TAMs) or myeloid derived suppressor cells (MDSCs) and methods of using the same, are disclosed.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(10) International Publication Number
WO 2018/195283 A1

(43) International Publication Date
25 October 2018 (25.10.2018)

(51) International Patent Classification:

C07K 16/28 (2006.01) *A61K 39/00* (2006.01)

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(21) International Application Number:

PCT/US2018/028315

(22) International Filing Date:

19 April 2018 (19.04.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/487,061 19 April 2017 (19.04.2017) US

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(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP,
KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,
SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a
patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii))

(54) Title: MULTISPECIFIC MOLECULES AND USES THEREOF

(57) Abstract: Multispecific molecules targeting tumor associated macrophages (TAMs) or myeloid derived suppressor cells (MDSCs) and methods of using the same, are disclosed.

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MULTISPECIFIC MOLECULES AND USES THEREOF

RELATED APPLICATION

This application claims priority to U.S. Serial No. 62/487061 filed April 19, 2017, the content of which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 18, 2018, is named E2070-7005WO_SL.txt and is 158,210 bytes in size.

BACKGROUND

Multispecific molecules targeting tumor associated macrophages (TAMs) or myeloid derived suppressor cells (MDSCs) and methods of using the same, are disclosed.

SUMMARY OF THE INVENTION

The disclosure relates, *inter alia*, to novel multispecific molecules comprising: (i) a first immunosuppressive myeloid cell (IMC) binding moiety (e.g., a first tumor associated macrophage (TAM) binding moiety; or a first myeloid derived suppressor cell (MDSC) binding moiety) (e.g., an antibody molecule); and (ii) a second IMC binding moiety (e.g., a first TAM binding moiety; or a second MDSC binding moiety) (e.g., an antibody molecule), wherein the first and the second IMC (e.g., TAM or MDSC) binding moieties are different. Without being bound by theory, the multispecific molecules disclosed herein are expected to deplete TAMs and/or MDSCs. Accordingly, provided herein are, *inter alia*, multispecific molecules (e.g., multispecific antibody molecules) that include the aforesaid moieties, nucleic acids encoding the same, methods of producing the aforesaid molecules, and methods of treating a cancer using the aforesaid molecules.

In one aspect, provided herein are isolated multispecific, e.g., a bispecific, molecules, comprising: (i) a first immunosuppressive myeloid cell (IMC) binding moiety (e.g., a first tumor

associated macrophage (TAM) binding moiety; or a first myeloid derived suppressor cell (MDSC) binding moiety) (e.g., an antibody molecule); and (ii) a second IMC binding moiety (e.g., a second TAM binding moiety; or a second MDSC binding moiety) (e.g., an antibody molecule), wherein the first and the second IMC (e.g., TAM or MDSC) binding moieties are different. In some embodiments, the first and the second IMC (e.g., TAM or MDSC) binding moieties bind to different epitopes. In some embodiments, the first and the second IMC (e.g., TAM or MDSC) binding moieties bind to different antigens.

In some embodiments, the first IMC binding moiety is a first MDSC binding moiety; and the second IMC binding moiety is a second MDSC binding moiety. In some embodiments, the first IMC binding moiety is a first TAM binding moiety; and the second IMC binding moiety is a second TAM binding moiety. In some embodiments, the first TAM binding moiety binds to CSF1R, CCR2, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1; and the second TAM binding moiety binds to CCR2, CSF1R, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1. In some embodiments, the first TAM binding moiety binds to CSF1R, CCR2, CXCR2, or PD-L1 (e.g., human CSF1R, CCR2, CXCR2, or PD-L1) and the second TAM binding moiety binds to CCR2, CSF1R, CXCR2, or PD-L1 (e.g., human CCR2, CSF1R, CXCR2, or PD-L1). In some embodiments, the first TAM binding moiety binds to CSF1R and the second TAM binding moiety binds to CCR2. In some embodiments, the first TAM binding moiety binds to CSF1R and the second TAM binding moiety binds to CXCR2. In some embodiments, the first TAM binding moiety binds to CCR2 and the second TAM binding moiety binds to CXCR2. In some embodiments, the first TAM binding moiety binds to CSF1R and the second TAM binding moiety binds to PD-L1. In some embodiments, the first TAM binding moiety binds to CCR2 and the second TAM binding moiety binds to PD-L1. In some embodiments, the first TAM binding moiety binds to CXCR2 and the second TAM binding moiety binds to PD-L1.

In some embodiments, the first TAM binding moiety binds to CSF1R, CCR2, CXCR2, or PD-L1 with a dissociation constant of less than about 10 nM, and more typically, 10 – 100 pM; and the second TAM binding moiety binds to CCR2, CSF1R, CXCR2, or PD-L1 with a dissociation constant of less than about 10 nM, and more typically, 10 – 100 pM. In some embodiments, the first TAM binding moiety binds to a conformational or a linear epitope on

CSF1R, CCR2, CXCR2, or PD-L1; and the second TAM binding moiety binds to a conformational or a linear epitope on CCR2, CSF1R, CXCR2, or PD-L1.

In some embodiments, the multispecific molecule comprises at least two non-contiguous polypeptide chains. In some embodiments, the first IMC binding moiety comprises a first anti-IMC antibody molecule and/or the second IMC binding moiety comprises a second anti-IMC antibody molecule. In some embodiments, the first anti-IMC antibody molecule and the second anti-IMC antibody molecule are, independently, a full antibody (e.g., an antibody that includes at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains), or an antigen-binding fragment (e.g., a Fab, F(ab')₂, Fv, a scFv, a single domain antibody, or a diabody (dAb)).

In some embodiments, the first anti-IMC antibody molecule and/or the second anti-IMC antibody molecule comprises a heavy chain constant region chosen from IgG1, IgG2, IgG3, or IgG4, or a fragment thereof.

In some embodiments, the first anti-IMC antibody molecule and/or the second anti-IMC antibody molecule comprises a light chain constant region chosen from the light chain constant regions of kappa or lambda, or a fragment thereof. In some embodiments, the first anti-IMC antibody molecule comprises a kappa light chain constant region, or a fragment thereof, and the second anti-IMC antibody molecule comprises a lambda light chain constant region, or a fragment thereof. In some embodiments, the first anti-IMC antibody molecule comprises a lambda light chain constant region, or a fragment thereof, and the second anti-IMC antibody molecule comprises a kappa light chain constant region, or a fragment thereof. In some embodiments, the first anti-IMC antibody molecule and the second anti-IMC antibody molecule have a common light chain variable region.

In some embodiments the multispecific molecule further comprises a heavy chain constant region (e.g., an Fc region) chosen from the heavy chain constant regions of IgG1, IgG2, and IgG4, more particularly, the heavy chain constant region of human IgG1, IgG2 or IgG4. In some embodiments, the heavy chain constant region (e.g., an Fc region) is linked to, e.g., covalently linked to, one or both of the first anti-IMC antibody molecule and the second anti-IMC antibody molecule. In some embodiments, the heavy chain constant region (e.g., an Fc region) is altered, e.g., mutated, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement

function. In some embodiments, an interface of a first and second heavy chain constant regions (e.g., Fc region) is altered, e.g., mutated, to increase or decrease dimerization, e.g., relative to a non-engineered interface. In some embodiments, the dimerization of the heavy chain constant region (e.g., Fc region) is enhanced by providing an Fc interface of a first and a second Fc region with one or more of: a paired cavity-protuberance (“knob-in-a hole”), an electrostatic interaction, or a strand-exchange, such that a greater ratio of heteromultimer:homomultimer forms, e.g., relative to a non-engineered interface. In some embodiments, the heavy chain constant region (e.g., Fc region) comprises an amino acid substitution at a position chosen from one or more of 347, 349, 350, 351, 366, 368, 370, 392, 394, 395, 397, 398, 399, 405, 407, or 409, e.g., of the Fc region of human IgG1, numbered based on the Eu numbering system. In some embodiments, the heavy chain constant region (e.g., Fc region) comprises an amino acid substitution chosen from: T366S, L368A, or Y407V (e.g., corresponding to a cavity or hole), or T366W (e.g., corresponding to a protuberance or knob), or a combination thereof, numbered based on the Eu numbering system.

In some embodiments, the heavy chain constant region (e.g., an Fc region) comprises one or more mutations that increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function, relative to a naturally-existing heavy chain constant region. In some embodiments, the first anti-IMC antibody molecule comprises a first heavy chain constant region (e.g., a first Fc region) and the second anti-IMC antibody molecule comprises a second heavy chain constant region (e.g., a second Fc region), wherein the first heavy chain constant region comprises one or more mutations that increase heterodimerization of the first heavy chain constant region and the second heavy chain constant region, relative to a naturally-existing heavy chain constant region, and/or wherein the second heavy chain constant region comprises one or more mutations that increase heterodimerization of the second heavy chain constant region and the first heavy chain constant region, relative to a naturally-existing heavy chain constant region. In some embodiments, the first and the second heavy chain constant regions (e.g., first and second Fc regions) comprise one or more of: a paired cavity-protuberance (“knob-in-a hole”), an electrostatic interaction, or a strand-exchange, such that a greater ratio of heteromultimer:homomultimer forms, e.g., relative to naturally-existing heavy chain constant regions. In some embodiments, the first and/or second heavy chain constant region (e.g., a first

and/or second Fc region, e.g., a first and/or second IgG1 Fc region) comprises an amino acid substitution at a position chosen from one or more of 347, 349, 350, 351, 366, 368, 370, 392, 394, 395, 397, 398, 399, 405, 407, or 409, numbered based on the Eu numbering system. In some embodiments, the first and/or second heavy chain constant region (e.g., a first and/or second Fc region, e.g., a first and/or second IgG1 Fc region) comprises an amino acid substitution chosen from: T366S, L368A, Y407V, or Y349C (e.g., corresponding to a cavity or hole), or T366W or S354C (e.g., corresponding to a protuberance or knob), or a combination thereof, numbered based on the Eu numbering system.

In some embodiments, the multispecific molecule further comprises a linker, e.g., a linker between one or more of: the first anti-IMC antibody molecule and the second anti-IMC antibody molecule, the first anti-IMC antibody molecule and the heavy chain constant region (e.g., the Fc region), or the second anti-IMC antibody molecule and the heavy chain constant region. In some embodiments, the linker is chosen from: a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker. In some embodiments, the linker is a peptide linker. In some embodiments, the peptide linker comprises Gly and Ser.

In some embodiments, the heavy chain constant region (e.g., Fc region) induces antibody dependent cellular cytotoxicity (ADCC).

In some embodiments, the first or the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 48, SEQ ID NO: 66, or SEQ ID NO: 69, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from a CDR of SEQ ID NO: 48, SEQ ID NO: 66, or SEQ ID NO: 69; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 50, SEQ ID NO: 67, or SEQ ID NO: 70, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from a CDR of SEQ ID NO: 50, SEQ ID NO: 67, or SEQ ID NO: 70. In some embodiments, the antibody molecule that binds to CSF1R comprises the heavy chain variable region sequence of: SEQ ID NO: 48, SEQ ID NO: 66, or SEQ ID NO: 69, or an amino acid sequence substantially identical thereto (e.g.,

95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 48, SEQ ID NO: 66, or SEQ ID NO: 69; and/or comprises the light chain variable region sequence of: SEQ ID NO: 50, SEQ ID NO: 67, or SEQ ID NO: 70, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 50, SEQ ID NO: 67, or SEQ ID NO: 70.

In some embodiments, the first or the second TAM binding moiety is an antibody molecule that binds to CCR2 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 64, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 64; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO: 63, SEQ ID NO: 65, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO: 63, SEQ ID NO: 65. In some embodiments, the antibody molecule that binds to CCR2 comprises the heavy chain variable region sequence of: SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 64, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 64; and/or comprises the light chain variable region sequence of: SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO: 63, SEQ ID NO: 65, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative

substitutions) to the amino acid sequence of SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO: 63, SEQ ID NO: 65.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 44, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 44; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 45, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 45; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 48, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 48; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 50, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 50.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 54, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 54; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 57, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 57; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 66, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*,

substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 66; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 67, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 67.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 54, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 54; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 57, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 57; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 69, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 69; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 70, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 70.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 59, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 59; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 60, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 60; and the second TAM binding moiety is an

antibody molecule that binds to CSF1R and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 66, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 66; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 67, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 67.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 59, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 59; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 60, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 60; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 69, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 69; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 70, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 70.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 62, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 62; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 63, or a

closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 63; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 66, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 66; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 67, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 67.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 62, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 62; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 63, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 63; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 69, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 69; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 70, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 70.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 64, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid

alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 64; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 65, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 65; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 66, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 66; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 67, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 67.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 64, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 64; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 65, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 65; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 69, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 69; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 70, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 70.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises the heavy chain variable region sequence of: SEQ ID NO: 44, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 44; and/or comprises the light chain variable region sequence of: SEQ ID NO: 45, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 45; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises the heavy chain variable region sequence of: SEQ ID NO: 48, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 48; and/or comprises the light chain variable region sequence of: SEQ ID NO: 50, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 50.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises the heavy chain variable region sequence of: SEQ ID NO: 54, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 54; and/or comprises the light chain variable region sequence of: SEQ ID NO: 57, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 57; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises the heavy chain variable region sequence of: SEQ ID NO: 66, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9%

identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 66; and/or comprises the light chain variable region sequence of: SEQ ID NO: 67, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 67.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises the heavy chain variable region sequence of: SEQ ID NO: 54, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 54; and/or comprises the light chain variable region sequence of: SEQ ID NO: 57, or a an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 57; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises the heavy chain variable region sequence of: SEQ ID NO: 69, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 69; and/or comprises the light chain variable region sequence of: SEQ ID NO: 70, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 70.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises the heavy chain variable region sequence of: SEQ ID NO: 59, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence

of SEQ ID NO: 59; and/or comprises the light chain variable region sequence of: SEQ ID NO: 60, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 60; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises the heavy chain variable region sequence of: SEQ ID NO: 66, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 66; and/or comprises the light chain variable region sequence of: SEQ ID NO: 67, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 67.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises the heavy chain variable region sequence of: SEQ ID NO: 59, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 59; and/or comprises the light chain variable region sequence of: SEQ ID NO: 60, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 60; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises the heavy chain variable region sequence of: SEQ ID NO: 69, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 69; and/or comprises the light chain variable region sequence of: SEQ ID NO: 70, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than

five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 70.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises the heavy chain variable region sequence of: SEQ ID NO: 62, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 62; and/or comprises the light chain variable region sequence of: SEQ ID NO: 63, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 63; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises the heavy chain variable region sequence of: SEQ ID NO: 66, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 66; and/or comprises the light chain variable region sequence of: SEQ ID NO: 67, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 67.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises the heavy chain variable region sequence of: SEQ ID NO: 62, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 62; and/or comprises the light chain variable region sequence of: SEQ ID NO: 63, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 63; and the second TAM binding moiety is an antibody

molecule that binds to CSF1R and comprises the heavy chain variable region sequence of: SEQ ID NO: 69, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 69; and/or comprises the light chain variable region sequence of: SEQ ID NO: 70, an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 70.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises the heavy chain variable region sequence of: SEQ ID NO: 64, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 64; and/or comprises the light chain variable region sequence of: SEQ ID NO: 65, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 65; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises the heavy chain variable region sequence of: SEQ ID NO: 66, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 66; and/or comprises the light chain variable region sequence of: SEQ ID NO: 67, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 67.

In one embodiment, the first TAM binding moiety is an antibody molecule that binds to CCR2 and comprises the heavy chain variable region sequence of: SEQ ID NO: 64, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at

least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 64; and/or comprises the light chain variable region sequence of: SEQ ID NO: 65, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 65; and the second TAM binding moiety is an antibody molecule that binds to CSF1R and comprises the heavy chain variable region sequence of: SEQ ID NO: 69, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 69; and/or comprises the light chain variable region sequence of: SEQ ID NO: 70, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 70.

In some embodiments, the first or the second TAM binding moiety is an antibody molecule that binds to PD-L1 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 109, SEQ ID NO: 111, or SEQ ID NO: 113, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 109, SEQ ID NO: 111, or SEQ ID NO: 113; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 110, SEQ ID NO: 112, or SEQ ID NO: 114, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 110, SEQ ID NO: 112, or SEQ ID NO: 114. In some embodiments, the antibody molecule that binds to PD-L1 comprises the heavy chain variable region sequence of: SEQ ID NO: 109, SEQ ID NO: 111, or SEQ ID NO: 113, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative

substitutions) to the amino acid sequence of SEQ ID NO: 109, SEQ ID NO: 111, or SEQ ID NO: 113); and/or comprises the light chain variable region sequence of: SEQ ID NO: 110, SEQ ID NO: 112, or SEQ ID NO: 114, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 110, SEQ ID NO: 112, or SEQ ID NO: 114).

In some embodiments, (i) the first IMC binding moiety binds to a first antigen (e.g., CSF1R, CCR2, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1) monovalently, and/or (ii) the second IMC binding moiety binds to a second antigen (e.g., CCR2, CSF1R, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1) monovalently, wherein the first antigen is different from the second antigen.

In some embodiments, (i) the multispecific molecule binds to a first antigen (e.g., CSF1R, CCR2, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1) monovalently, and/or (ii) the multispecific molecule binds to a second antigen (e.g., CCR2, CSF1R, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1) monovalently, wherein the first antigen is different from the second antigen.

In some embodiments, (i) the multispecific molecule inhibits a first antigen in the presence of a second antigen, optionally wherein the multispecific molecule reduces an activity of the first antigen in a cell, e.g., by at least 40, 50, 60, 70, 80, or 90%, when the cell expresses both the first antigen and the second antigen on the cell surface, and/or (ii) the multispecific molecule does not inhibit or does not substantially inhibit the first antigen in the absence of the second antigen, optionally wherein the multispecific molecule does not reduce an activity of the first antigen, or does not reduce an activity of the first antigen by more than 2, 4, 6, 8, 10, or 15%, when the cell expresses the first antigen but not the second antigen on the cell surface.

In some embodiments, (i) the multispecific molecule inhibits a second antigen in the presence of a first antigen, optionally wherein the multispecific molecule reduces an activity of the second antigen in a cell, e.g., by at least 40, 50, 60, 70, 80, or 90%, when the cell expresses both the first antigen and the second antigen on the cell surface, and/or (ii) the multispecific

molecule does not inhibit or does not substantially inhibit the second antigen in the absence of the first antigen, optionally wherein the multispecific molecule does not reduce an activity of the second antigen, or does not reduce an activity of the second antigen by more than 2, 4, 6, 8, 10, or 15%, when the cell expresses the second antigen but not the first antigen on the cell surface.

In some embodiments, the multispecific molecule further comprises one or more additional binding moieties (e.g., a third binding moiety, a fourth binding moiety, (e.g., a trispecific or a tetraspecific molecule). In some embodiments, the multispecific molecule further comprises one or more additional binding moieties (e.g., a third binding moiety, a fourth binding moiety, (e.g., a trispecific or a tetraspecific molecule). In some embodiments, the multispecific molecule comprises a third TAM binding moiety (e.g., an antibody molecule), wherein the third TAM binding moiety is different from the first and the second TAM binding moieties. In some embodiments, the first TAM binding moiety binds to human CSF1R, the second TAM binding moiety binds to human CCR2, and the third TAM binding moiety binds to CXCR2.

In some embodiments, the multispecific molecule comprises a third binding moiety (e.g., antibody molecule) that is a tumor targeting moiety. In some embodiments, the tumor targeting moiety binds to PD-L1, mesothelin, CD47, ganglioside 2 (GD2), prostate stem cell antigen (PSCA), prostate specific membrane antigen (PMSA), prostate-specific antigen (PSA), carcinoembryonic antigen (CEA), Ron Kinase, c-Met, Immature laminin receptor, TAG-72, BING-4, Calcium-activated chloride channel 2, Cyclin-B1, 9D7, Ep-CAM, EphA3, Her2/neu, Telomerase, SAP-1, Survivin, NY-ESO-1/LAGE-1, PRAME, SSX-2, Melan-A/MART-1, Gp100/pmell17, Tyrosinase, TRP-1/-2, MC1R, β -catenin, BRCA1/2, CDK4, CML66, Fibronectin, p53, Ras, TGF-B receptor, AFP, ETA, MAGE, MUC-1, CA-125, BAGE, GAGE, NY-ESO-1, β -catenin, CDK4, CDC27, CD47, α actinin-4, TRP1/gp75, TRP2, gp100, Melan-A/MART1, gangliosides, WT1, EphA3, Epidermal growth factor receptor (EGFR), CD20, MART-2, MART-1, MUC1, MUC2, MUM1, MUM2, MUM3, NA88-1, NPM, OA1, OGT, RCC, RUI1, RUI2, SAGE, TRG, TRP1, TSTA, Folate receptor alpha, L1-CAM, CAIX, EGFRvIII, gpA33, GD3, GM2, VEGFR, Integrins (Integrin α V β 3, Integrin α 5 β 1), Carbohydrates (Le), IGF1R, EPHA3, TRAILR1, TRAILR2, or RANKL.

In some embodiments, the multispecific molecule is a bispecific molecule comprising a first and a second non-contiguous polypeptides, wherein: (i) the first polypeptide includes, e.g., in the N- to C-orientation, the first TAM binding moiety (e.g., an antibody molecule (e.g., a first

portion of a first antigen domain, e.g., a first VH-CH1 of a Fab molecule)), that binds to, e.g., a first TAM antigen, e.g., CSF1R, CCR2, CXCR2, or PD-L1, connected, optionally via a linker to, a first domain that promotes association between the first and the second polypeptide (e.g., a first immunoglobulin constant domain (e.g., a first Fc molecule as described herein); (ii) the second polypeptide includes, e.g., in the N- to C-orientation, the second TAM binding moiety (e.g., an antibody molecule, e.g., a scFv that binds to, e.g., a second TAM antigen, e.g., CCR2, CSF1R, CXCR2, or PD-L1)), connected, optionally, via a linker to, a second domain that promotes association between the first and the second polypeptide (e.g., a second immunoglobulin constant domain (e.g., a second Fc molecule as described herein); and (iii) the third polypeptide includes, e.g., in the N- to C-orientation, a second portion of the first antigen domain, e.g., a first VL-CL of the Fab, that binds to the first TAM antigen, e.g., wherein the third polypeptide associates non-covalently to the first polypeptide; and (iv) the fourth polypeptide includes, e.g., in the N- to C-orientation, a second portion of the second antigen domain, e.g., a second VL-CL of the Fab, that binds to the second TAM antigen, e.g., wherein the fourth polypeptide associates non-covalently to the second polypeptide. In some embodiments, the first and the second polypeptides are homo- or heterodimers.

In some embodiments, the multispecific molecule is a bispecific molecule, wherein:

(i) the first TAM binding moiety (e.g., a binding moiety that binds to a first TAM antigen, e.g., CSF1R, CCR2, or CXCR2) comprises a first and a second non-contiguous polypeptides, and

(ii) the second TAM binding moiety (e.g., a binding moiety that binds to a second TAM antigen, e.g., CSF1R, CCR2, or CXCR2) comprises a third and a fourth non-contiguous polypeptides, wherein:

(a) the first polypeptide comprises, e.g., in the N- to C-orientation, a first VH, a first CH1, connected, optionally via a linker, to a first domain (e.g., a first Fc region) that promotes association between the first and the third polypeptides,

(b) the second polypeptide comprises, e.g., in the N- to C-orientation, a first VL and a first CL,

(c) the third polypeptide comprises, e.g., in the N- to C-orientation, a second VH, a second CH1, connected, optionally via a linker, to a second domain (e.g., a second Fc region) that promotes association between the first and the third polypeptides, and

(d) the fourth polypeptide comprises, e.g., in the N- to C-orientation, a second VL and a second CL. In some embodiments, the first and the second domains (e.g., the first and the second Fc regions) form a homo- or heterodimer.

In one aspect, the invention provides an isolated multispecific, e.g., a bispecific, molecule, comprising (i) an anti-CSF1R binding moiety (e.g., an anti-CSF1R antibody molecule); and (ii) an anti-CCR2 binding moiety (e.g., an anti-CCR2 antibody molecule). Without wishing to be bound by theory, the anti-CSF1R/anti-CCR2 multispecific molecule may preferentially bind to a CSF1R-positive, CCR2-positive cell relative to a CSF1R-positive, CCR2-negative cell, or a CSF1R-negative, CCR2-positive cell. Exemplary CSF1R-positive, CCR2-positive cell include, but are not limited to, tumor-associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs). Exemplary CSF1R-positive, CCR2-negative cells include, but are not limited to, tissue-resident macrophages (e.g., Kupffer cells), and Langerhans cells. Exemplary CSF1R-negative, CCR2-positive cells include, but are not limited to, T cells (e.g., activated T cells, e.g., activated CD4+ and/or CD8+ T cells), NK cells, and neutrophils. Without wishing to be bound by theory, the anti-CSF1R/anti-CCR2 multispecific molecule may preferentially bind to CSF1R-positive, CCR2-positive cells (e.g., pro-tumorigenic TAMs or MDSCs) relative to CSF1R-positive, CCR2-negative cells (e.g., tissue-resident macrophages (e.g., Kupffer cells), or Langerhans cells), or CSF1R-negative, CCR2-positive cells (e.g., activated T cells or NK cells).

In some embodiments, the anti-CSF1R/anti-CCR2 multispecific molecule, when it binds to a target cell, may induce antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) of the target cell. In some embodiments, the anti-CSF1R/anti-CCR2 multispecific molecule may preferentially bind to and reduce the number of immunosuppressive myeloid cells in the tumor microenvironment (e.g., TAMs or MDSCs), while sparing homeostatic myeloid cells (e.g., tissue-resident macrophages (e.g., Kupffer cells)) and other anti-tumor immune cells (e.g., activated T cells and NK cells). Depletion of homeostatic myeloid cells may be partially responsible for adverse events in patients receiving anti-CSF1R antibody therapies.

In some embodiments, the multispecific molecule has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or more) of the following properties:

(i) the multispecific molecule preferentially binds to a CSF1R-positive, CCR2-positive cell relative to a CSF1R-positive, CCR2-negative cell, or a CSF1R-negative, CCR2-positive cell, e.g., the binding of the multispecific molecule to the CSF1R-positive, CCR2-positive cell is at least 2, 4, 6, 8, 10, 15, 20, or 25-fold stronger than the binding of the multispecific molecule to the CSF1R-positive, CCR2-negative cell, or the CSF1R-negative, CCR2-positive cell, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 2 with respect to FIG. 1;

(ii) the multispecific molecule preferentially binds to a CSF1R-positive, CCR2-positive cell relative to a CSF1R-positive, CCR2-negative cell, e.g., the EC50 of the multispecific molecule for binding to a CSF1R-positive, CCR2-positive cell is no more than 60, 50, 40, 30, 20, or 10 % of the EC50 of the multispecific molecule for binding to a CSF1R-positive, CCR2-negative cell, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 2 with respect to FIG. 1;

(iii) the multispecific molecule preferentially binds to a CSF1R-positive, CCR2-positive cell relative to a CSF1R-negative, CCR2-positive cell, e.g., the EC50 of the multispecific molecule for binding to a CSF1R-positive, CCR2-positive cell is no more than 50, 40, 30, 20, 10, or 5% of the EC50 of the multispecific molecule for binding to a CSF1R-negative, CCR2-positive cell, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 2 with respect to FIG. 1;

(iv) the multispecific molecule preferentially binds to tumor-associated macrophages (TAMs) or myeloid derived suppressor cells (MDSCs) relative to T cells, NK cells, neutrophils, tissue-resident macrophages (e.g., Kupffer cells), or Langerhans cells, e.g., the binding of the multispecific molecule to TAMs or MDSCs is at least 2, 4, 6, 8, 10, 15, 20, or 25-fold stronger than the binding of the multispecific molecule to T cells, NK cells, neutrophils, tissue-resident macrophages (e.g., Kupffer cells), or Langerhans cells, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 6 with respect to FIG. 5;

(v) the multispecific molecule inhibits monocyte migration, e.g., monocyte chemoattractant protein 1 (MCP1)-induced monocyte migration, e.g., reduces MCP1-induced monocyte migration by at least 40, 50, 60, or 70%, e.g., as measured using a transwell plate migration assay, e.g., as measured using methods described in Example 3 with respect to FIG. 2;

(vi) the multispecific molecule inhibits the proliferation of macrophages, e.g., bone marrow-derived macrophages, e.g., CSF-1-induced proliferation of bone marrow-derived macrophages, e.g., reduces CSF-1-induced proliferation of bone marrow-derived macrophages by at least 50, 60, 70, or 80%, e.g., as measured using a cell proliferation MTT assay, e.g., as measured using methods described in Example 4 with respect to FIG. 3B;

(vii) the multispecific molecule does not inhibit or does not substantially inhibit the differentiation of monocytes, e.g., bone marrow-derived monocytes, e.g., CSF-1-induced differentiation of bone marrow-derived monocytes, e.g., does not reduce CSF-1-induced differentiation of bone marrow-derived monocytes by more than 2, 4, 6, 8, or 10%, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 5 with respect to FIG. 4;

(viii) the multispecific molecule depletes suppressive myeloid cells, e.g., TAMs or MDSCs, e.g., reduces the number of suppressive myeloid cells, e.g., TAMs or MDSCs, by at least 80, 85, 90, 95, 99, or 99.5%, in vivo, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 7 with respect to FIG. 6;

(ix) the multispecific molecule does not deplete or does not substantially deplete tissue-resident macrophages, e.g., Kupffer cells, e.g., does not reduce the number of tissue-resident macrophages, e.g., Kupffer cells, by more than 4, 6, 8, 10, or 15%, in vivo, e.g., as measured using an immunohistochemistry analysis, e.g., as measured using methods described in Example 8 with respect to FIGs. 7B and 7D;

(x) the multispecific molecule does not inhibit or does not substantially inhibit CSF-1 dependent cell survival of CSF1R-positive, CCR2-negative cells, e.g., does not reduce CSF-1 dependent cell survival of CSF1R-positive, CCR2-negative cells by more than 5, 10, or 15%, e.g., as measured using a cell viability MTT assay, e.g., as measured using methods described in Example 9 with respect to FIG. 8A;

(xi) the multispecific molecule increases CD8+ T cell tumor infiltration in vivo, e.g., increases % CD8+ T cells in CD3+ T cells in tumor by at least 1.5, 2, or 2.5-fold, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 10 with respect to FIG. 9;

(xii) the multispecific molecule reduces Treg frequency in tumor in vivo, e.g., reduces Treg frequency in tumor by at least 15, 20, 25, or 30%, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 11 with respect to FIG. 10A;

(xiii) the multispecific molecule increases the CD8+ T cell/Treg ratio in tumor in vivo, e.g., increases the CD8+ T cell/Treg ratio in tumor by at least 2.5, 3, 3.5, 4, or 4.5-fold, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 11 with respect to FIG. 10B; or

(xiv) the multispecific molecule reduces tumor growth and/or increases survival of a tumor-bearing animal, e.g., as measured using methods described in Example 12 with respect to FIGs. 11A and 11B.

In some embodiments, the multispecific molecule preferentially binds to a CSF1R-positive, CCR2-positive cell relative to a CSF1R-positive, CCR2-negative cell, or a CSF1R-negative, CCR2-positive cell, e.g., the binding of the multispecific molecule to the CSF1R-positive, CCR2-positive cell is at least 2, 4, 6, 8, 10, 15, 20, or 25-fold stronger than the binding of the multispecific molecule to the CSF1R-positive, CCR2-negative cell, or the CSF1R-negative, CCR2-positive cell, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 2 with respect to FIG. 1.

In some embodiments, the multispecific molecule preferentially binds to a CSF1R-positive, CCR2-positive cell relative to a CSF1R-positive, CCR2-negative cell, e.g., the EC50 of the multispecific molecule for binding to a CSF1R-positive, CCR2-positive cell is no more than 60, 50, 40, 30, 20, or 10 % of the EC50 of the multispecific molecule for binding to a CSF1R-positive, CCR2-negative cell, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 2 with respect to FIG. 1.

In some embodiments, the multispecific molecule preferentially binds to a CSF1R-positive, CCR2-positive cell relative to a CSF1R-negative, CCR2-positive cell, e.g., the EC50 of the multispecific molecule for binding to a CSF1R-positive, CCR2-positive cell is no more than 50, 40, 30, 20, 10, or 5% of the EC50 of the multispecific molecule for binding to a CSF1R-negative, CCR2-positive cell, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 2 with respect to FIG. 1.

In some embodiments, the multispecific molecule preferentially binds to tumor-associated macrophages (TAMs) or myeloid derived suppressor cells (MDSCs) relative to T

cells, NK cells, neutrophils, tissue-resident macrophages (e.g., Kupffer cells), or Langerhans cells, e.g., the binding of the multispecific molecule to TAMs or MDSCs is at least 2, 4, 6, 8, 10, 15, 20, or 25-fold stronger than the binding of the multispecific molecule to T cells, NK cells, neutrophils, tissue-resident macrophages (e.g., Kupffer cells), or Langerhans cells, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 6 with respect to FIG. 5.

In some embodiments, the multispecific molecule inhibits monocyte migration, e.g., monocyte chemoattractant protein 1 (MCP1)-induced monocyte migration, e.g., reduces MCP1-induced monocyte migration by at least 40, 50, 60, or 70%, e.g., as measured using a transwell plate migration assay, e.g., as measured using methods described in Example 3 with respect to FIG. 2.

In some embodiments, the multispecific molecule inhibits the proliferation of macrophages, e.g., bone marrow-derived macrophages, e.g., CSF-1-induced proliferation of bone marrow-derived macrophages, e.g., reduces CSF-1-induced proliferation of bone marrow-derived macrophages by at least 50, 60, 70, or 80%, e.g., as measured using a cell proliferation MTT assay, e.g., as measured using methods described in Example 4 with respect to FIG. 3B.

In some embodiments, the multispecific molecule does not inhibit or does not substantially inhibit the differentiation of monocytes, e.g., bone marrow-derived monocytes, e.g., CSF-1-induced differentiation of bone marrow-derived monocytes, e.g., does not reduce CSF-1-induced differentiation of bone marrow-derived monocytes by more than 2, 4, 6, 8, or 10%, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 5 with respect to FIG. 4.

In some embodiments, the multispecific molecule depletes suppressive myeloid cells, e.g., TAMs or MDSCs, e.g., reduces the number of suppressive myeloid cells, e.g., TAMs or MDSCs, by at least 80, 85, 90, 95, 99, or 99.5%, in vivo, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 7 with respect to FIG. 6.

In some embodiments, the multispecific molecule does not deplete or does not substantially deplete tissue-resident macrophages, e.g., Kupffer cells, e.g., does not reduce the number of tissue-resident macrophages, e.g., Kupffer cells, by more than 4, 6, 8, 10, or 15%, in

vivo, e.g., as measured using an immunohistochemistry analysis, e.g., as measured using methods described in Example 8 with respect to FIGs. 7B and 7D.

In some embodiments, the multispecific molecule does not inhibit or does not substantially inhibit CSF-1 dependent cell survival of CSF1R-positive, CCR2-negative cells, e.g., does not reduce CSF-1 dependent cell survival of CSF1R-positive, CCR2-negative cells by more than 5, 10, or 15%, e.g., as measured using a cell viability MTT assay, e.g., as measured using methods described in Example 9 with respect to FIG. 8A.

In some embodiments, the multispecific molecule increases CD8+ T cell tumor infiltration in vivo, e.g., increases % CD8+ T cells in CD3+ T cells in tumor by at least 1.5, 2, or 2.5-fold, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 10 with respect to FIG. 9.

In some embodiments, the multispecific molecule reduces Treg frequency in tumor in vivo, e.g., reduces Treg frequency in tumor by at least 15, 20, 25, or 30%, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 11 with respect to FIG. 10A.

In some embodiments, the multispecific molecule increases the CD8+ T cell/Treg ratio in tumor in vivo, e.g., increases the CD8+ T cell/Treg ratio in tumor by at least 2.5, 3, 3.5, 4, or 4.5-fold, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 11 with respect to FIG. 10B.

In some embodiments, the multispecific molecule reduces tumor growth and/or increases survival of a tumor-bearing animal, e.g., as measured using methods described in Example 12 with respect to FIGs. 11A and 11B.

In some embodiments, the anti-CSF1R binding moiety and the anti-CCR2 binding moiety are, independently, a full antibody (e.g., an antibody that includes at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains), or an antigen-binding fragment (e.g., a Fab, F(ab')₂, Fv, a scFv, a single domain antibody, or a diabody (dAb)). In some embodiments, the anti-CSF1R binding moiety and/or the anti-CCR2 binding moiety comprises a heavy chain constant region chosen from IgG1, IgG2, IgG3, or IgG4, or a fragment thereof. In some embodiments, the anti-CSF1R binding moiety and/or the anti-CCR2 binding moiety comprises a heavy chain constant region that can mediate antibody-dependent

cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). In some embodiments, the anti-CSF1R binding moiety comprises a first heavy chain constant region (e.g., a first Fc region) and the anti-CCR2 binding moiety comprises a second heavy chain constant region (e.g., a second Fc region), wherein the first heavy chain constant region comprises one or more mutations that increase heterodimerization of the first heavy chain constant region and the second heavy chain constant region, relative to a naturally-existing heavy chain constant region, and/or wherein the second heavy chain constant region comprises one or more mutations that increase heterodimerization of the second heavy chain constant region and the first heavy chain constant region, relative to a naturally-existing heavy chain constant region. In some embodiments, the anti-CSF1R binding moiety and/or the anti-CCR2 binding moiety comprises a light chain constant region chosen from the light chain constant regions of kappa or lambda, or a fragment thereof. In some embodiments, the anti-CSF1R binding moiety comprises a kappa light chain constant region, or a fragment thereof, and the anti-CCR2 binding moiety comprises a lambda light chain constant region, or a fragment thereof. In some embodiments, the anti-CSF1R binding moiety comprises a lambda light chain constant region, or a fragment thereof, and the anti-CCR2 binding moiety comprises a kappa light chain constant region, or a fragment thereof. In some embodiments, the anti-CSF1R binding moiety and the anti-CCR2 binding moiety have a common light chain variable region. In some embodiments, the multispecific molecule further comprises a heavy chain constant region (e.g., an Fc region) chosen from the heavy chain constant regions of IgG1, IgG2, and IgG4, more particularly, the heavy chain constant region of human IgG1, IgG2 or IgG4. In some embodiments, the multispecific molecule further comprises a heavy chain constant region that can mediate antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).

In some embodiments, the multispecific molecule comprises an anti-CSF1R antibody molecule and an anti-CCR2 antibody molecule, wherein:

(i) the anti-CSF1R antibody molecule comprises a first polypeptide and a second polypeptide, wherein the first polypeptide comprises a first light chain variable region (VL) and a first light chain constant region (CL), and the second polypeptide comprises a first heavy chain variable region (VH), a first heavy chain constant region 1 (CH1), and optionally, a first CH2 and a first CH3, and

(ii) the anti-CCR2 antibody molecule comprises a third polypeptide and a fourth polypeptide, wherein the third polypeptide comprises a second VL and a second CL, and the fourth polypeptide comprises a second VH, a second CH1, and optionally, a second CH2 and a second CH3.

In some embodiments, (i) the anti-CSF1R antibody molecule binds to CSF1R monovalently, and/or (ii) the anti-CCR2 antibody molecule binds to CCR2 monovalently. In some embodiments, the multispecific molecule binds to CSF1R monovalently, and/or binds to CCR2 monovalently. In some embodiments, the multispecific molecule binds to CSF1R monovalently, and binds to CCR2 monovalently.

In some embodiments,

(i) the multispecific molecule inhibits CSF1R in the presence of CCR2, optionally wherein the multispecific molecule reduces an activity of CSF1R (e.g., CSF1R signaling, e.g., CSF1-induced CSF1R signaling) in a cell, e.g., by at least 40, 50, 60, 70, 80, or 90%, when the cell expresses both CSF1R and CCR2 on the cell surface, and/or

(ii) the multispecific molecule does not inhibit or does not substantially inhibit CSF1R in the absence of CCR2, optionally wherein the multispecific molecule does not reduce an activity of CSF1R (e.g., CSF1R signaling, e.g., CSF1-induced CSF1R signaling), or does not reduce an activity of CSF1R by more than 2, 4, 6, 8, 10, or 15%, when the cell expresses CSF1R but not CCR2 on the cell surface.

In some embodiments,

(i) the multispecific molecule inhibits CCR2 in the presence of CSF1R, optionally wherein the multispecific molecule reduces an activity of CCR2 in a cell, e.g., by at least 40, 50, 60, 70, 80, or 90%, when the cell expresses both CCR2 and CSF1R on the cell surface, and/or

(ii) the multispecific molecule does not inhibit or does not substantially inhibit CCR2 in the absence of CSF1R, optionally wherein the multispecific molecule does not reduce an activity of CCR2, or does not reduce an activity of CCR2 by more than 2, 4, 6, 8, 10, or 15%, when the cell expresses CCR2 but not CSF1R on the cell surface.

In one aspect, this invention provides an isolated multispecific, e.g., a bispecific, molecule, comprising:

(i) a first binding moiety that binds to a molecule that mediates the trafficking of monocytes, e.g., inflammatory monocytes, e.g., Ly6C^{Hi} CCR2^+ $\text{CX3CR1}^{\text{Lo}}$ inflammatory monocytes, optionally wherein the first binding moiety binds to CCR2; and

(ii) a second binding moiety that binds to a molecule that mediates the maturation and/or survival of monocytes and/or macrophages at an inflamed tissue, optionally wherein the second binding moiety binds to CSF1R.

In one aspect, this invention provides an isolated multispecific, e.g., a bispecific, molecule, comprising:

(i) a first binding moiety that reduces recruitment of inflammatory monocytes to tumor, optionally wherein the first binding moiety binds to and/or inhibits CCR2; and

(ii) a second binding moiety that reduces maturation and/or survival of monocytes and/or macrophages in the tumor microenvironment, optionally wherein the second binding moiety binds to and/or inhibits CSF1R.

In one aspect, this invention provides an isolated multispecific, e.g., a bispecific, molecule, comprising (i) an anti-CSF1R binding moiety (e.g., an anti-CSF1R antibody molecule); and (ii) an anti-PD-L1 binding moiety (e.g., an anti-PD-L1 antibody molecule).

In some embodiments of the aforementioned aspects and embodiments, the multispecific molecule further comprises one or more additional binding moieties (e.g., a third binding moiety, a fourth binding moiety, (e.g., a trispecific or a tetraspecific molecule), optionally wherein the third binding moiety is a third IMC binding moiety or a tumor targeting moiety. In some embodiments, the tumor targeting moiety is a tumor targeting moiety disclosed in WO2017165464, e.g., pages 108-118 of WO2017165464, herein incorporated by reference in its entirety.

In some embodiments of the aforementioned aspects and embodiments, the multispecific molecule further comprises an immune cell engager chosen from a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager. In some embodiment, the immune cell engager is an immune cell engager disclosed in WO2017165464, e.g., pages 119-131 of WO2017165464, herein incorporated by reference in its entirety. In some embodiments, the immune cell engager binds to and activates an immune cell, e.g., an effector

cell. In some embodiments, the immune cell engager binds to, but does not activate, an immune cell, e.g., an effector cell.

In some embodiments, the immune cell engager is a T cell engager, e.g., a T cell engager that mediates binding to and activation of a T cell, or a T cell engager that mediates binding to but not activation of a T cell. In some embodiments, the T cell engager binds to CD3, TCR α , TCR β , TCR γ , TCR ζ , ICOS, CD28, CD27, HVEM, LIGHT, CD40, 4-1BB, OX40, DR3, GITR, CD30, TIM1, SLAM, CD2, or CD226, e.g., the T cell engager is an anti-CD3 antibody molecule.

In some embodiments, the immune cell engager is an NK cell engager, e.g., an NK cell engager that mediates binding to and activation of an NK cell, or an NK cell engager that mediates binding to but not activation of an NK cell. In some embodiments, the NK cell engager is chosen from an antibody molecule, e.g., an antigen binding domain, or ligand that binds to (e.g., activates): NKp30, NKp40, NKp44, NKp46, NKG2D, DNAM1, DAP10, CD16 (e.g., CD16a, CD16b, or both), CRTAM, CD27, PSGL1, CD96, CD100 (SEMA4D), NKp80, CD244 (also known as SLAMF4 or 2B4), SLAMF6, SLAMF7, KIR2DS2, KIR2DS4, KIR3DS1, KIR2DS3, KIR2DS5, KIR2DS1, CD94, NKG2C, NKG2E, or CD160.

In some embodiments, the immune cell engager is a B cell engager, e.g., a CD40L, an OX40L, or a CD70 ligand, or an antibody molecule that binds to OX40, CD40 or CD70. In some embodiments, the immune cell engager is a macrophage cell engager, e.g., a CD2 agonist; a CD40L; an OX40L; an antibody molecule that binds to OX40, CD40 or CD70; an agonist of a Toll-like receptor (TLR) (e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4) or a TLR9 agonist); CD47; or a STING agonist.

In some embodiments, the immune cell engager is a dendritic cell engager, e.g., a CD2 agonist, an OX40 antibody, an OX40L, 41BB agonist, a Toll-like receptor agonist or a fragment thereof (e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4)), CD47 agonist, or a STING agonist.

In some embodiments of the aforementioned aspects and embodiments, the multispecific molecule further comprises a cytokine molecule. In some embodiments, the cytokine molecule is chosen from interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-12 (IL-12), interleukin-15 (IL-15), interleukin-18 (IL-18), interleukin-21 (IL-21), or interferon gamma, or a fragment or variant thereof, or a combination of any of the aforesaid cytokines. In some embodiments, the

cytokine molecule is a cytokine molecule disclosed in WO2017165464, e.g., pages 108-118 of WO2017165464, herein incorporated by reference in its entirety.

In some embodiments of the aforementioned aspects and embodiments, the multispecific molecule further comprises a stromal modifying moiety. In some embodiments, the stromal modifying moiety causes one or more of: decreases the level or production of a stromal or extracellular matrix (ECM) component; decreases tumor fibrosis; increases interstitial tumor transport; improves tumor perfusion; expands the tumor microvasculature; decreases interstitial fluid pressure (IFP) in a tumor; or decreases or enhances penetration or diffusion of an agent, e.g., a cancer therapeutic or a cellular therapy, into a tumor or tumor vasculature. In some embodiments, the stromal modifying moiety is a stromal modifying moiety disclosed in WO2017165464, e.g., pages 131-136 of WO2017165464, herein incorporated by reference in its entirety.

In another aspect, provided herein are isolated nucleic acid molecules encoding the multispecific molecule (e.g., antibody) of any one of the preceding claims.

In another aspect, provided herein are isolated nucleic acid molecules, which comprises the nucleotide sequence encoding any of the multispecific molecules described herein, or a nucleotide sequence substantially homologous thereto (e.g., at least 95% to 99.9% identical thereto).

In another aspect, provided herein are vectors, e.g., expression vectors, comprising one or more of the nucleic acid molecules described herein.

In another aspect, provided herein are cells, e.g., host cells, comprising the nucleic acid molecule described herein or the vector described herein.

In another aspect, provided herein are methods of making, e.g., producing, the multispecific molecules described herein, comprising culturing the cell, e.g., the host cell, described herein, under suitable conditions, e.g., conditions suitable for gene expression and/or heterodimerization.

In another aspect, provided herein are pharmaceutical compositions comprising the multispecific molecule described herein and a pharmaceutically acceptable carrier, excipient, or stabilizer.

In another aspect, provided herein are methods of treating a hyperproliferative disorder, a cancer, a fibrotic disorder or condition, an inflammatory disorder or condition, or an autoimmune disorder. In one embodiment, the disorder is a hyperproliferative disorder, e.g., a hyperproliferative connective tissue disorder (e.g., a hyperproliferative fibrotic disease). In one embodiment, the fibrotic (e.g., hyperproliferative fibrotic) disease is multisystemic or organ-specific. Exemplary fibrotic diseases include, but are not limited to, multisystemic (e.g., systemic sclerosis, multifocal fibrosclerosis, sclerodermatous graft-versus-host disease in bone marrow transplant recipients, nephrogenic systemic fibrosis, scleroderma), and organ-specific disorders (e.g., fibrosis of the lung, liver, heart, kidney, pancreas, skin and other organs). In other embodiments, the fibrotic disease is chosen from liver fibrosis (e.g., liver cirrhosis, NASH, and other conditions described herein), pulmonary fibrosis (e.g., IPF), renal fibrosis, or fibrosis of the bone marrow (e.g., myelofibrosis).

In another aspect, provided herein are methods of treating a cancer in a subject, comprising administering to the subject in need thereof the multispecific molecule described herein, wherein the multispecific molecule is administered in an amount effective to treat the cancer.

In another aspect, provided herein are method of treating a cancer in a subject, comprising administering to the subject in need thereof the multispecific molecule described herein, wherein the multispecific molecule is administered in an amount effective to reduce the number of TAMs (e.g., the number of TAMs in or near a tumor in the subject), inhibit the proliferation of TAMs (e.g., the proliferation of TAMs in or near a tumor in the subject), or reduce or inhibit macrophage infiltration into a tumor in the subject.

In another aspect, provided herein are methods of treating a cancer in a subject by reducing a portion of a population of TAMs, comprising administering to the subject in need thereof the multispecific molecule described herein, wherein the multispecific molecule is administered in an amount effective to inhibit or deplete a portion of the population of TAMs.

In another aspect, provided herein are methods of reducing the proliferation of a portion of a population of TAMs in a subject (e.g., in a subject having cancer, e.g., a solid tumor), comprising, administering to the subject in need thereof the multispecific molecule described

herein, wherein the multispecific molecule is administered in an amount effective to reduce the proliferation of a portion of the population of TAMs.

In another aspect, provided herein are methods of inhibiting or depleting a portion of a population of TAMs in a subject having a cancer (e.g., a tumor), comprising administering to the subject the multispecific molecule described herein, wherein the multispecific molecule is administered in an amount effective to reduce the number of tumor infiltrating macrophages, inhibit the proliferation of tumor infiltrating macrophages, or reduce macrophage infiltration into a tumor.

In some embodiments, the cancer is a solid tumor cancer or a metastatic lesion. In some embodiments, the solid tumor cancer is one or more of pancreatic cancer (e.g., pancreatic adenocarcinoma), breast cancer, colorectal cancer, lung cancer (e.g., small or non-small cell lung cancer), skin cancer (e.g., melanoma), ovarian cancer, liver cancer, or brain cancer (e.g., glioma). In some embodiments, the cancer is characterized as containing TAMs, is associated with the presence of TAMs, TAMs are in and/or form part of the cancer (e.g., tumor), or TAMs have been detected in or near the solid tumor.

In some embodiments, the methods further comprise identifying the presence of TAMs in or near the cancer (e.g., tumor) in the subject. In some embodiments, the TAMs express CXCR2 and CCR2, CCR2 and CSF1R, CSF1R and CXCR2, or CCR2, CXCR2, and CSF1R.

In some embodiments, the methods further comprise administering a second therapeutic treatment. In some embodiments, the second therapeutic treatment comprises a therapeutic agent (e.g., a chemotherapeutic agent, a biologic agent, hormonal therapy), radiation, or surgery. In some embodiments, the therapeutic agent is selected from: a chemotherapeutic agent, or a biologic agent. In some embodiments, the therapeutic agent is a checkpoint inhibitor. In some embodiments, the check point inhibitor is selected from the group consisting of an anti-CTLA4 antibody, an anti-PD1 antibody (e.g., Nivolumab, Pembrolizumab or Pidilizumab), an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-CD160 antibody, an anti-2B4 antibody, an anti-CD80 antibody, an anti-CD86 antibody, an anti-B7-H3 (CD276) antibody, an anti-B7-H4 (VTCN1) antibody, an anti-HVEM (TNFRSF14 or CD270) antibody, an anti-BTLA antibody, an anti-KIR antibody, an anti-MHC class I antibody, an anti-MHC class II antibody, an anti-GAL9 antibody, an anti-VISTA antibody, an anti-BTLA antibody, an anti-TIGIT antibody, an anti-LAIR1 antibody, and an anti-A2aR antibody.

In another aspect, provided herein are methods of treating a cancer in a subject, comprising administering to the subject in need thereof the multispecific molecule described herein, wherein the multispecific molecule is administered in an amount effective to reduce the number of MDSCs (e.g., the number of MDSCs in or near a tumor in the subject), inhibit the proliferation of MDSCs (e.g., the number of MDSCs in or near a tumor in the subject), or reduce or inhibit MDSC infiltration into a tumor in the subject.

In another aspect, provided herein are methods of treating a cancer in a subject by reducing a portion of a population of MDSCs, comprising administering to the subject in need thereof the multispecific molecule described herein, wherein the multispecific molecule is administered in an amount effective to inhibit or deplete a portion of the population of MDSCs.

In another aspect, provided herein are methods of reducing the proliferation of a portion of a population of MDSCs in a subject (e.g., in a subject having cancer, e.g., a solid tumor), comprising, administering to the subject in need thereof the multispecific molecule described herein, wherein the multispecific molecule is administered in an amount effective to reduce the proliferation of a portion of the population of MDSCs.

In another aspect, provided herein are methods of inhibiting or depleting a portion of a population of MDSCs in a subject having a cancer (e.g., a tumor), comprising administering to the subject the multispecific molecule described herein, wherein the multispecific molecule is administered in an amount effective to reduce the number of MDSCs, inhibit the proliferation of MDSCs, or reduce MDSC infiltration into a tumor.

In some embodiments, the cancer is a solid tumor cancer or a metastatic lesion. In some embodiments, the solid tumor cancer is one or more of pancreatic cancer (e.g., pancreatic adenocarcinoma), breast cancer, colorectal cancer, lung cancer (e.g., small or non-small cell lung cancer), skin cancer (e.g., melanoma), ovarian cancer, liver cancer, or brain cancer (e.g., glioma). In some embodiments, the cancer is characterized as containing MDSCs, is associated with the presence of MDSCs, MDSCs are in and/or form part of the cancer (e.g., tumor), or MDSCs have been detected in or near the solid tumor. In some embodiments, the methods further comprise identifying the presence of MDSCs in or near the cancer (e.g., tumor) in the subject.

In some embodiments, the methods further comprise administering a second therapeutic treatment. In some embodiments, the second therapeutic treatment comprises a therapeutic agent

(e.g., a chemotherapeutic agent, a biologic agent, hormonal therapy), radiation, or surgery. In some embodiments, the therapeutic agent is selected from: a chemotherapeutic agent, or a biologic agent. In some embodiments, the therapeutic agent is a checkpoint inhibitor. In some embodiments, the check point inhibitor is selected from the group consisting of an anti-CTLA4 antibody, an anti-PD1 antibody (e.g., Nivolumab, Pembrolizumab or Pidilizumab), an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-CD160 antibody, an anti-2B4 antibody, an anti-CD80 antibody, an anti-CD86 antibody, an anti-B7-H3 (CD276) antibody, an anti-B7-H4 (VTCN1) antibody, an anti-HVEM (TNFRSF14 or CD270) antibody, an anti-BTLA antibody, an anti-KIR antibody, an anti-MHC class I antibody, an anti-MHC class II antibody, an anti-GAL9 antibody, an anti-VISTA antibody, an anti-BTLA antibody, an anti-TIGIT antibody, an anti-LAIR1 antibody, and an anti-A2aR antibody.

In one aspect, provided herein are methods of treating a fibrotic disease or disorder in a subject in need thereof, comprising administering to the subject an effective amount of a multispecific molecule disclosed herein, thereby treating the fibrotic disease or disorder. In some embodiments, the fibrotic disease or disorder is a fibrotic disease or disorder of the lung, the liver, the heart or vasculature, the kidney, the pancreas, the skin, the gastrointestinal tract, the bone marrow or a hematopoietic tissue, the nervous system, the eye, or a combination thereof. In some embodiments, the fibrotic disease or disorder is lung fibrosis (e.g., Idiopathic pulmonary fibrosis (IPF)) or liver fibrosis (e.g., Nonalcoholic steatohepatitis (NASH)).

In some embodiments, treatment of a fibrotic condition includes reducing or inhibiting one or more of: formation or deposition of tissue fibrosis; reducing the size, cellularity (e.g., fibroblast or immune cell numbers), composition; or cellular content, of a fibrotic lesion; reducing the collagen or hydroxyproline content, of a fibrotic lesion; reducing expression or activity of a fibrogenic protein; reducing fibrosis associated with an inflammatory response; decreasing weight loss associated with fibrosis; or increasing survival.

In some embodiments, the fibrotic disease or disorder is primary fibrosis. In one embodiment, the fibrotic disease or disorder is idiopathic. In other embodiments, the fibrotic disease or disorder is associated with (e.g., is secondary to) a disease (e.g., an infectious disease, an inflammatory disease, an autoimmune disease, a malignant or cancerous disease, and/or a connective disease); a toxin; an insult (e.g., an environmental hazard (e.g., asbestos, coal dust,

polycyclic aromatic hydrocarbons), cigarette smoking, a wound); a medical treatment (e.g., surgical incision, chemotherapy or radiation), or a combination thereof.

In one aspect, provided herein are methods of treating a liver disease or disorder in a subject in need thereof, comprising administering to the subject an effective amount of a multispecific molecule disclosed herein, thereby treating the liver disease or disorder. In some embodiments, the liver disease or disorder is a fibrotic disorder or connective tissue disorder affecting the function or physiology of the liver. In one embodiment, the fibrotic disorder or connective tissue disorder can be systemic (affecting the whole body), multi-organ, or organ-specific (e.g., liver-specific). Examples of fibrotic liver disorders include, but are not limited to, liver fibrosis (hepatic fibrosis), liver cirrhosis, and any disorder associated with accumulation of extracellular matrix proteins, e.g., collagen, in the liver, liver scarring, and/or abnormal hepatic vasculature. In one embodiment, the liver disease or disorder is liver cirrhosis. Liver cirrhosis is considered to be an end stage of liver fibrosis, involving regenerative nodules (as a result of repair processes), and is typically accompanied with the distortion of the hepatic vasculature. In other embodiments, the liver disease or disorder is a liver cancer. Examples of liver cancers include, but are not limited to, hepatocellular carcinoma (HCC), primary liver cell carcinoma, hepatoma, fibrolamellar carcinoma, focal nodular hyperplasia, cholangiosarcoma, intrahepatic bile duct cancer, angiosarcoma or hemangiosarcoma, hepatic adenoma, hepatic hemangiomas, hepatic hamartoma, hepatoblastoma, infantile hemangioendothelialoma, mixed tumors of the liver, tumors of mesenchymal tissue, and sarcoma of the liver. Liver cancers can also be associated with metastasis of non-liver cancers, such as breast cancer, colorectal cancer, esophageal cancer, kidney or renal cancer, lung cancer, ovarian cancer, pancreatic cancer, rectal cancer, skin cancer (e.g., melanoma), gastric or stomach cancer (including gastrointestinal cancer), and uterine cancer. In one embodiment, the liver disease or disorder is HCC. In certain embodiments, the liver disease or disorder is caused by one or more insults including, but not limited to, liver inflammation or damage; viral (e.g., chronic viral) infection (e.g., hepatitis B, hepatitis C virus, hepatitis A virus, hepatitis D virus (hepatitis delta virus), hepatitis E virus, Epstein-Barr adenovirus, or cytomegalovirus; or parasitic infection, such as schistosomiasis); alcoholism; fatty liver disease; metabolic disorders (e.g., hemochromatosis, diabetes, obesity, hypertension, dyslipidemia, galactosemia, or glycogen storage disease); autoimmune disorders

(e.g., autoimmune hepatitis (AIH), autoimmune liver disease, lupoid hepatitis, systemic lupus erythematosus, primary biliary cirrhosis (PBC), scleroderma, or systemic sclerosis); inflammatory liver disorders (e.g., steatohepatitis, primary sclerosing cholangitis (PSC), ulcerative colitis, Crohn's disease, inflammatory bowel disease); inherited or congenital liver disease (e.g., Wilson's disease, Gilbert's disease, Byler syndrome, Greenland-Eskimo familial cholestasis, Zellweger's syndrome, Alagilles syndrome (ALGS), progressive familial intrahepatic cholestasis (PFIC), alpha 1-antitrypsin deficiency, cystic fibrosis, Indian childhood cirrhosis, or hereditary hemochromatosis); and liver injury (e.g., drug toxicity, alcoholism, ischemia, malnutrition, or physical trauma). In one embodiment, the liver disease or disorder is fatty liver (or FLD), alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcoholic steatohepatitis, simple steatosis, Reye's syndrome, and any disorder associated with abnormal retention of lipids in liver cells.

In one aspect, provided herein are methods of treating an inflammatory disorder or condition in a subject in need thereof, comprising administering to the subject an effective amount of a multispecific molecule disclosed herein, thereby treating the inflammatory disorder or condition. In one embodiment, the inflammatory disorder or condition is an inflammatory disorder or condition in the kidney. In one embodiment, the inflammatory disorder or condition is Lupus nephritis.

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. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. In vitro binding of increasing concentrations of UniTI-01 to transiently transfected ExpiCHO cells expressing mCCR2 (circles), mCSF1R (squares), or both mCCR2 and mCSF1R (triangles), as determined by flow analysis. In FIG. 1, percent fluorescence (%) is plotted against antibody concentrations tested.

FIG. 2. Effect of increasing concentrations of isotype control (mIgG2a), anti-mouse CCR2 monoclonal antibody (aCCR2) or UniTI-01 on MCP-1-dependent cell migration of bone marrow-derived monocytes in a trans-well cell culture system.

FIGs. 3A and 3B. Effect of increasing concentrations of UniTI-01, anti-mouse CSF1R monoclonal antibody (aCSF1R), or isotype control (mIgG2a) on mCSF-1-dependent differentiation and proliferation of bone marrow-derived monocytes into macrophages in vitro. FIG. 3A is a pair of flow cytometry plots showing the staining of bone marrow cells with an anti-CCR2 antibody and an anti-CSF1R antibody at day 0 or day 4 of cell culture in the presence of mCSF1. In FIG. 3B, % proliferation is plotted for each condition tested. All antibodies were tested at 15 µg/ml.

FIG. 4. Effect of UniTI-01, anti-mouse CSF1R monoclonal antibody (aCSF1R), or isotype control (mIgG2a) on mCSF-1-dependent differentiation and proliferation of bone marrow-derived monocytes. All antibodies were tested at 15 µg/ml.

FIG. 5. In vitro binding of increasing concentrations of UniTI-01 to primary intratumoral M-MDSCs and M2-like macrophages, as determined by flow analysis. FIG. 5 is a panel of histograms showing staining of CD206+ macrophages, M-MDSCs, neutrophils, or CD3+ T cells using UniTI-01.

FIG. 6. Effect of in vivo administration of UniTI-01 on intratumoral myeloid cell populations in EMT6 and MC38 syngeneic tumor models.

FIGs. 7A-7D. Effect of in vivo administration of UniTI-01 on Kupffer cells in EMT6 syngeneic tumor model. FIGs. 7A and 7B are immunohistochemistry graphs of tumor and liver tissues, respectively, stained with the antibody F4/80. FIGs. 7C and 7D are graphs showing % F4/80 positive area for tumor and liver tissues, respectively.

FIGs. 8A and 8B. Effect of UniTI-01 on CSF-1 dependent cell survival in CCR2-negative NFS-60 cells in vitro. In FIG. 8A, cell viability is plotted against the antibody concentrations tested for the anti-CCR2/anti-CSF1R bispecific antibody UniTI-01, a bivalent

monospecific anti-CSF1R antibody (aCSF1R), or a monovalent monospecific anti-CSF1R antibody (mono-aCSF1R). FIG. 8B is a pair of flow cytometry plots showing the staining of NFS-60 cells with an anti-CCR2 antibody and an anti-CSF1R antibody, or the staining of NFS-60 cells with an isotype control antibody.

FIG. 9. Effect of in vivo administration of UniTI-01 on CD8+ T cell infiltration in the tumor of EMT6 model. FIG. 9 is a graph showing % CD8+ T cells in CD3+ T cells in tumors under indicated treatments.

FIGs. 10A and 10B. Effect of in vivo administration of UniTI-01 on Treg frequency in the tumor of MC38 model. FIG. 10A is a graph showing % FOXP3+ cells in CD4+ T cells in tumors under indicated treatments. In FIG. 10B, the CD8+ T cell/Treg ratio in tumors is plotted for each treatment.

FIGs. 11A and 11B. UniTI-01 shows antitumor efficacy, tumor regressions and enhanced survival when treated in combination with anti-PDL1 antibody in EMT6 tumor model. FIG. 11A is a panel of graphs showing tumor volumes for each treatment. FIG. 11B is a graph showing percent survival under indicated treatments.

DETAILED DESCRIPTION OF THE INVENTION

TAMs originate from circulating monocytes and their recruitment into tumors is driven by tumor-derived chemotactic factors. TAMs can promote tumor cell proliferation and metastasis by causing such responses as inhibition of B and T cell activation, inhibition of tumor-associated antigen presentation, inhibition of cytotoxic granule release, increased angiogenesis, and secretion a wide range of growth and proangiogenic factors (*see e.g.*, Liu *et al* Cellular & Molecular Immunology (2015) 12, 1–4; and Noy, Roy *et al* Immunity, Volume 41 , Issue 1 , 49–61; and Quatromoni *et al.* Am J Transl Res. 2012; 4(4): 376–389). Consequently, many tumors with a high number of TAMs have an increased tumor growth rate, local proliferation and distant metastasis. Thus, therapies that deplete TAMs or inhibit their activity would be useful.

Certain terms are defined below.

As used herein, the term “variant” of a parent sequence refers to a sequence that has a substantially identical amino acid sequence to the parent sequence, or a fragment thereof. In some embodiments, the variant is a functional variant.

As used herein, the articles “a” and “an” refer to one or more than one, *e.g.*, to at least one, of the grammatical object of the article. The use of the words “a” or “an” when used in conjunction with the term “comprising” herein may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

As used herein, “about” and “approximately” generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given range of values.

“Antibody molecule” as used herein refers to a protein, *e.g.*, an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable region sequence. An antibody molecule encompasses antibodies (*e.g.*, full-length antibodies) and antibody fragments. In an embodiment, an antibody molecule comprises an antigen binding or functional fragment of a full length antibody, or a full length immunoglobulin chain. For example, a full-length antibody is an immunoglobulin (Ig) molecule (*e.g.*, an IgG antibody) that is naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes). In embodiments, an antibody molecule refers to an immunologically active, antigen-binding portion of an immunoglobulin molecule, such as an antibody fragment. An antibody fragment, *e.g.*, functional fragment, is a portion of an antibody, *e.g.*, Fab, Fab', F(ab')₂, F(ab)₂, variable fragment (Fv), domain antibody (dAb), or single chain variable fragment (scFv). A functional antibody fragment binds to the same antigen as that recognized by the intact (*e.g.*, full-length) antibody. The terms “antibody fragment” or “functional fragment” also include isolated fragments consisting of the variable regions, such as the “Fv” fragments consisting of the variable regions of the heavy and light chains or recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker (“scFv proteins”). In some embodiments, an antibody fragment does not include portions of antibodies without antigen binding activity, such as Fc fragments or single amino acid residues. Exemplary antibody molecules include full length antibodies and antibody fragments, *e.g.*, dAb (domain antibody), single chain, Fab, Fab', and F(ab')₂ fragments, and single chain variable fragments (scFvs).

As used herein, an “immunoglobulin variable region sequence” refers to an amino acid sequence which can form the structure of an immunoglobulin variable region. For example, the sequence may include all or part of the amino acid sequence of a naturally-occurring variable region. For example, the sequence may or may not include one, two, or more N- or C-terminal amino acids, or may include other alterations that are compatible with formation of the protein structure.

In embodiments, an antibody molecule is monospecific, e.g., it comprises binding specificity for a single epitope. In some embodiments, an antibody molecule is multispecific, e.g., it comprises a plurality of immunoglobulin variable region sequences, where a first immunoglobulin variable region sequence has binding specificity for a first epitope and a second immunoglobulin variable region sequence has binding specificity for a second epitope. In some embodiments, an antibody molecule is a bispecific antibody molecule. “Bispecific antibody molecule” as used herein refers to an antibody molecule that has specificity for more than one (e.g., two, three, four, or more) epitope and/or antigen.

“Antigen” (Ag) as used herein refers to a molecule that can provoke an immune response, e.g., involving activation of certain immune cells and/or antibody generation. Any macromolecule, including almost all proteins or peptides, can be an antigen. Antigens can also be derived from genomic recombinant or DNA. For example, any DNA comprising a nucleotide sequence or a partial nucleotide sequence that encodes a protein capable of eliciting an immune response encodes an “antigen.” In embodiments, an antigen does not need to be encoded solely by a full length nucleotide sequence of a gene, nor does an antigen need to be encoded by a gene at all. In embodiments, an antigen can be synthesized or can be derived from a biological sample, e.g., a tissue sample, a tumor sample, a cell, or a fluid with other biological components. As used, herein a “tumor antigen” or interchangeably, a “cancer antigen” includes any molecule present on, or associated with, a cancer, e.g., a cancer cell or a tumor microenvironment that can provoke an immune response. As used, herein an “immune cell antigen” includes any molecule present on, or associated with, an immune cell that can provoke an immune response.

The “antigen-binding site,” or “binding portion” of an antibody molecule refers to the part of an antibody molecule, e.g., an immunoglobulin (Ig) molecule, that participates in antigen binding. In embodiments, the antigen binding site is formed by amino acid residues of the variable (V) regions of the heavy (H) and light (L) chains. Three highly divergent stretches

within the variable regions of the heavy and light chains, referred to as hypervariable regions, are disposed between more conserved flanking stretches called “framework regions,” (FRs). FRs are amino acid sequences that are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In embodiments, in an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface, which is complementary to the three-dimensional surface of a bound antigen. The three hypervariable regions of each of the heavy and light chains are referred to as “complementarity-determining regions,” or “CDRs.” The framework region and CDRs have been defined and described, e.g., in Kabat, E.A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917. Each variable chain (e.g., variable heavy chain and variable light chain) is typically made up of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the amino acid order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

“Cancer” as used herein can encompass all types of oncogenic processes and/or cancerous growths. In embodiments, cancer includes primary tumors as well as metastatic tissues or malignantly transformed cells, tissues, or organs. In embodiments, cancer encompasses all histopathologies and stages, e.g., stages of invasiveness/severity, of a cancer. In embodiments, cancer includes relapsed and/or resistant cancer. The terms “cancer” and “tumor” can be used interchangeably. For example, both terms encompass solid and liquid tumors. As used herein, the term “cancer” or “tumor” includes premalignant, as well as malignant cancers and tumors.

The compositions and methods of the present invention encompass polypeptides and nucleic acids having the sequences specified, or sequences substantially identical or similar thereto, e.g., sequences at least 85%, 90%, 95% identical or higher to the sequence specified. In the context of an amino acid sequence, the term “substantially identical” is used herein to refer to a first amino acid that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a

common structural domain having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *e.g.*, a sequence provided herein.

In the context of nucleotide sequence, the term “substantially identical” is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity. For example, nucleotide sequences having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *e.g.*, a sequence provided herein.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid “identity” is equivalent to amino acid or nucleic acid “homology”).

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch ((1970) *J. Mol. Biol.* 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a

Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used unless otherwise specified) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller ((1989) *CABIOS*, 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a “query sequence” to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid (e.g., SEQ ID NO: 1) molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

It is understood that the molecules of the present invention may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on their functions.

The term “amino acid” is intended to embrace all molecules, whether natural or synthetic, which include both an amino functionality and an acid functionality and capable of being included in a polymer of naturally-occurring amino acids. Exemplary amino acids include naturally-occurring amino acids; analogs, derivatives and congeners thereof; amino acid analogs

having variant side chains; and all stereoisomers of any of any of the foregoing. As used herein the term “amino acid” includes both the D- or L- optical isomers and peptidomimetics.

A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

The terms “polypeptide”, “peptide” and “protein” (if single chain) are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component. The polypeptide can be isolated from natural sources, can be a produced by recombinant techniques from a eukaryotic or prokaryotic host, or can be a product of synthetic procedures.

The terms “nucleic acid,” “nucleic acid sequence,” “nucleotide sequence,” or “polynucleotide sequence,” and “polynucleotide” are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. The polynucleotide may be either single-stranded or double-stranded, and if single-stranded may be the coding strand or non-coding (antisense) strand. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. The nucleic acid may be a recombinant polynucleotide, or a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which either does not occur in nature or is linked to another polynucleotide in a non-natural arrangement.

The term “isolated,” as used herein, refers to material that is removed from its original or native environment (*e.g.*, the natural environment if it is naturally occurring). For example, a

naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated by human intervention from some or all of the co-existing materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of the environment in which it is found in nature.

As used herein, the term “immunosuppressive myeloid cell” or “IMC” generally refers to a cell of myeloid lineage that promotes immunosuppression (e.g., in a tumor microenvironment) (e.g., by inhibiting T cell activation, inhibiting T cell viability, promoting T regulatory cell induction and recruitment). Immunosuppressive myeloid cells include, e.g., tumor associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs).

As used herein, the term “tumor associated macrophage” or “TAM” generally refers to a macrophage that exists in the microenvironment of a cancer, for example, a tumor.

As used herein, the term “reducing TAMs” generally refers to decreasing the number of TAMs. Reducing includes decreasing the number of TAMs in a tumor or near a tumor (e.g., as compared to the number of TAMs prior to administration of a multispecific molecule described herein (e.g., prior to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more administrations of a multispecific molecule described herein). Reducing includes decreasing any number of TAMs (e.g., 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100%, all, or substantially) (e.g., as compared to the number of TAMs prior to administration of a multispecific molecule described herein (e.g., prior to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more administrations of a multispecific molecule described herein).

As used herein, the term “myeloid derived suppressor cell” or “MDSC” generally refers to a cell of myeloid origin that is capable of promoting immunosuppression and commonly express CD33, CD11b and CD45. Various subpopulations of MDSCs have been defined, for example monocytic-MDSCs (M-MDSCs) are commonly associated with expression of CD14 and CD124 and low expression of HLA-DR. In some embodiments, the MDSC population is an MO-MDSC population. Polymorphonuclear MDSCs (PMN-MDSCs) are associated with expression of CD15, CD66b, and CD124, and no expression of HLA-DR. Immature MDSCs (I-MDSCs) are associated with expression of CD117 and CD34 and no expression of LIN and HLA-DR. See e.g., Ugel et al. (2015) JCI Vol 125 (9), page 3365.

As used herein, the term “a CSF1R-positive, CCR2-positive cell” refers to a cell expressing both CSF1R and CCR2 on the cell surface. The term “a CSF1R-positive, CCR2-negative cell” refers to a cell expressing CSF1R, but not CCR2 on the cell surface. The term “a CSF1R-negative, CCR2-positive cell” refers to a cell expressing CCR2, but not CSF1R on the cell surface.

As used herein, a binding moiety, e.g., an antibody molecule, binds to a target monovalently, when the binding moiety, e.g., the antibody molecule, binds to a single epitope on the target. In some embodiments, the binding moiety comprises only one antigen binding domain to the target. In some embodiments, one molecule of the binding moiety can only bind to one molecule of the target.

As used herein, a binding moiety, e.g., an antibody molecule, binds to a target bivalently, when the binding moiety, e.g., the antibody molecule, binds to two epitopes on the target. In some embodiments, the two epitopes are identical. In some embodiments, the two epitopes are different. In some embodiments, the binding moiety comprises two antigen binding domains to the target. In some embodiments, one molecule of the binding moiety can bind to two molecules of the target.

Various aspects of the invention are described in further detail below. Additional definitions are set out throughout the specification.

Antigens

TAM targeting antigens of the present disclosure include, e.g., CSF1R, CCR2, CXCR2, CD68, CD163, CX3CR1, MARCO, CD204, CD52, and folate receptor beta. Exemplary amino acid sequences of TAM targeting antigens are provided herein.

CSF1R

CSF1R (also known as Macrophage colony-stimulating factor 1 receptor) is a tyrosine-protein kinase that acts as cell-surface receptor for CSF1 and IL34 and plays an essential role in the regulation of survival, proliferation and differentiation of hematopoietic precursor cells, especially mononuclear phagocytes, such as macrophages and monocytes. CSF1R promotes the release of pro-inflammatory chemokines in response to IL34 and CSF1, and thereby plays an

important role in innate immunity and in inflammatory processes. Exemplary CSF1R immature amino acid sequences are provided in SEQ ID NOs: 87 and 88.

CSF1R immature amino acid sequence isoform 1 (identifier: P07333-1):

MGPGLVLLLLLVATAWHGQGIPVIEPSVPELVVKPGATVTLRCVGNGSVEWDGPPSPHWTLYSDG
 SSSILSTNNATFQNTGTYRCTEPGDPLGGSAAIHLYVKDPAWPWNVLAQEVVVFEDQDALLPCL
 LTDPVLEAGVSLVRVRGRPLMRHTNYSFSPWHGFTIHRAKFIQSQDYQCSALMGGRKVMSSISIR
 LKVQKVIPGPPALTLVPAELVRIRGEAAQIVCSASSVDVNFDFVFLQHNNTKLAIPQQSDFHNNR
 YQKVLTLNLDQVDFQHAGNYSVASNVQKGHSTSMFFRVVESAYLNLSSSEQNLIQEVTVGEGLN
 LKVMVEAYPGLQGFNWTYLGPFSDHQPEPKLANATTKDTYRHTFTLSLPRLKPSEAGRYSFLAR
 NPGGWRALTFELTLRYPPEVSVIWFINGSGTLLCAASGYPPQPNVTWLQCSGHTDRCDEAQVLQ
 VWDDPYPEVLSQEPFHKVTVQSLLTVETLEHNQTYECRAHNSVSGSGSWAFIPISAGATHPPDE
 FLFTPVVVACMSIMALLLLLLLLLLLYKYKQKPKYQVRWKIIESYEGNSYTFIDPTQLPYNEKWE
 FPRNNLQFGKTLGAGAFGKVVEATAFGLGKEDAVLKVAVKMLKSTAHADKEKEALMSELKIMSHL
 GQHENIVNLLGACTHGGPVLVITEYCCYGDLLNFLRRKAEAMLGPSLSPGQDPEGGVVDYKNIHL
 EKKYVRRDSGFSQGVDTYVEMRPVSTSSNDSFSEQDLKEDGRPLELRDLLHFSSQVAQGMFAF
 LASKNCIHRDVAARNVLLTNGHVAKIGDFGLARDIMNDSNYIVKGNARLPVKWMAPESEIFDCVY
 TVQSDVWSYGILLWEIFSLGLNPYPGILVNSKFYKLVKDGYPMAQPAFAPKNIYSIMQACWALE
 PTHRPTFQQICSFLLQEQAQEDRRERDYNLPSSSRSGSGSGSSSELEEESSSEHLTCCEQGDIA
 QPLLQPNNYQFC

SEQ ID NO: 87

CSF1R immature amino acid sequence isoform 2 (identifier: P07333-2):

MGPGLVLLLLLVATAWHGQGIPVIEPSVPELVVKPGATVTLRCVGNGSVEWDGPPSPHWTLYSDG
 SSSILSTNNATFQNTGTYRCTEPGDPLGGSAAIHLYVKDPAWPWNVLAQEVVVFEDQDALLPCL
 LTDPVLEAGVSLVRVRGRPLMRHTNYSFSPWHGFTIHRAKFIQSQDYQCSALMGGRKVMSSISIR
 LKVQKVIPGPPALTLVPAELVRIRGEAAQIVCSASSVDVNFDFVFLQHNNTKLAIPQQSDFHNNR
 YQKVLTLNLDQVDFQHAGNYSVASNVQKGHSTSMFFRVVGTPSPSLCPA

SEQ ID NO: 88

CCR2

CCR2 (also known as C-C chemokine receptor type 2) is a G protein coupled receptor for the CCL2, CCL7 and CCL13 chemokines. CCR2 is known to function in the recruitment of monocytes/macrophages and T cells. CCR2 is expressed is expressed on monocytes and a small subpopulation of T cells and exhibits an almost identical expression pattern in mice and humans (Mack et al. J Immunol 2001; 166:4697-4704). Exemplary CCR2 amino acid sequences are provided in SEQ ID NOs: 89 and 90.

CCR2 amino acid sequence isoform A (Identifier: P41597-1):

MLSTSRSRFIRNTNESGEEVTTFFDYDYGAPCHKFDVKQIGAQLLPPLYSLVFIFGFVGN
 MLVVLILINCKKLKCLTDIYLLNLAI SDLLFLITLPLWAHSAANEWVFGNAMCKLFTGLY
 HIGYFGGIFFIILLTIDRYLAIVHAVFALKARTVTFGVVTSVITWLVAVFASVPGIIFTK
 CQKEDSVYVCGPYFPRGWNNFHTIMRNILGLVLP LLIMVICYSGILKTLLRCRNEKKRHR
 AVRVIFTIMIVYFLFWTPYNIVILLNTFQEFFGLSNCESTSQLDQATQVTETLGMTHCCI
 NP IIYAFVGEKF RSLFHIALGCRIAPLQKPVC GPGVRPGKNVKVTTQGLLDGRGKGKSI
 GRAPEASLQDKEGA

SEQ ID NO: 89

CCR2 amino acid sequence isoform B (Identifier: P41597-2):

MLSTSRSRFIRNTNESGEEVTTFFDYDYGAPCHKFDVKQIGAQLLPPLYSLVFIFGFVGN
 MLVVLILINCKKLKCLTDIYLLNLAI SDLLFLITLPLWAHSAANEWVFGNAMCKLFTGLY
 HIGYFGGIFFIILLTIDRYLAIVHAVFALKARTVTFGVVTSVITWLVAVFASVPGIIFTK
 CQKEDSVYVCGPYFPRGWNNFHTIMRNILGLVLP LLIMVICYSGILKTLLRCRNEKKRHR
 AVRVIFTIMIVYFLFWTPYNIVILLNTFQEFFGLSNCESTSQLDQATQVTETLGMTHCCI
 NP IIYAFVGEKF RRYLSVFFRKHITKRFC KQCPVFYRETVDGVTSTNTPSTGEQEV SAGL

SEQ ID NO: 90

CXCR2

CXCR2 (also known as interleukin-8 receptor) is the G protein coupled receptor for IL8 which is a neutrophil chemotactic factor. Binding of IL8 to the receptor causes activation of neutrophils. This response is mediated via a G-protein that activates a phosphatidylinositol-calcium second messenger system. CXCR2 binds to IL-8 with high affinity, and also binds with high affinity to CXCL3, GRO/MGSA and NAP-2. CXCR2 is expressed at high levels on circulating neutrophils and is critical for directing their migration to sites of inflammation (J Clin Invest. 2012;122(9):3127-3144). An exemplary CXCR2 amino acid sequence is provided in SEQ ID NO: 91.

CXCR2 amino acid sequence (Identifier: P25025-1):

MEDFNMESDSFEDFWKGEDLSNYSYSTLP PFLLD AAPCEPESLEINKYFVVIIYALVFL
 LSLLGNSLVMLVILYSRVGRSVTDVYLLNLALADLLFALTLP IWAASKVNGWIFGTFLCKVVSL
 LKEVNFYSGILL LACISVD RYLAIVHATRTL TQKRYLVKFICLSIWGLSLLLALPV
 LLFRRTVYSSNVSPACYEDMGNNTANWRMLLRILPQSFGFIVP LLIMLFCYGF TLRTL FK
 AHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLLADTL MRTQVIQETCERNHIDRALDATEILGI
 LHSCLNPLIYAFIGQKFRHGLLKILAIHGLISKDSL PKDSRPSFVGSSSGHTSTTL

SEQ ID NO: 91

Exemplary Antibodies

Exemplary antibodies binding TAM antigens are provided throughout the specification and below. Exemplary anti-CSF1R antibodies are described herein as well as in WO2009026303A1; WO2011123381A1; WO2016207312A1; WO2016106180A1; US20160220669A1; US20160326254A1; WO2013169264A1; WO2013087699A1; WO2011140249A2; WO2011131407A1; WO2011123381A1; WO2011107553A1; and WO2011070024A1, all of which are herein incorporated by reference in their entirety. Exemplary CCR2 antibodies are described herein as well as in WO2013192596A2; WO2010021697A2; WO2001057226A1; and WO1997031949A1, all of which are herein incorporated by reference in their entirety. Exemplary CXCR2 antibodies are described in WO2014170317A1 and US20160060347 (see e.g., a) SEQ ID NO: 14 (light chain) and SEQ ID NO: 15 (heavy chain); b) SEQ ID NO: 24 (light chain) and SEQ ID NO: 25 (heavy chain); c) SEQ ID NO: 34 (light chain) and SEQ ID NO: 35 (heavy chain); d) SEQ ID NO: 44 (light chain) and SEQ ID NO: 45 (heavy chain); e) SEQ ID NO: 54 (light chain) and SEQ ID NO: 55 (heavy chain); f) SEQ ID NO: 64 (light chain) and SEQ ID NO: 65 (heavy chain); g) SEQ ID NO: 74 (light chain) and SEQ ID NO: 75 (heavy chain); h) SEQ ID NO: 84 (light chain) and SEQ ID NO: 85 (heavy chain)), all of which are herein incorporated by reference in their entirety. Exemplary anti-CD163 antibodies are provided in US20120258107 (see e.g., MAC2158, MAC2-48), herein incorporated by reference in its entirety. Exemplary anti-CD52 antibodies are described in US20050152898, herein incorporated by reference in its entirety. Exemplary anti-folate antibodies are described in US9522196, herein incorporated by reference in its entirety. Exemplary anti-CD52 antibodies are described in US20050152898, herein incorporated by reference in its entirety. Exemplary anti-MARCO antibodies are described in WO2016196612, herein incorporated by reference in its entirety.

Antibody Molecules

In one embodiment, the multispecific molecule comprises an antibody molecule that binds to a first tumor associated macrophage (TAM) antigen; and an antibody molecule that binds to a second TAM antigen. In some embodiments, the first and/or second TAM antigen is, e.g., a mammalian, e.g., a human. For example, the antibody molecule binds specifically to an epitope, e.g., linear or conformational epitope, on the TAM antigen.

In one embodiment, the multispecific molecule comprises an antibody molecule that binds to a first myeloid derived suppressor cell (MDSC) antigen; and an antibody molecule that binds to a second MDSC antigen. In some embodiments, the first and/or second MDSC antigen is, *e.g.*, a mammalian, *e.g.*, a human. For example, the antibody molecule binds specifically to an epitope, *e.g.*, linear or conformational epitope, on the MDSC antigen.

In an embodiment, an antibody molecule is a monospecific antibody molecule and binds a single epitope. *E.g.*, a monospecific antibody molecule having a plurality of immunoglobulin variable region sequences, each of which binds the same epitope.

In an embodiment an antibody molecule is a multispecific antibody molecule, *e.g.*, it comprises a plurality of immunoglobulin variable region sequences, wherein a first immunoglobulin variable region sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable region sequence of the plurality has binding specificity for a second epitope. In an embodiment the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment the first and second epitopes overlap. In an embodiment the first and second epitopes do not overlap. In an embodiment the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment a multispecific antibody molecule comprises a third, fourth or fifth immunoglobulin variable region. In an embodiment, a multispecific antibody molecule is a bispecific antibody molecule, a trispecific antibody molecule, or a tetraspecific antibody molecule.

In an embodiment a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable region sequence which has binding specificity for a first epitope and a second immunoglobulin variable region sequence that has binding specificity for a second epitope. In an embodiment the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment the first and second epitopes overlap. In an embodiment the first and second epitopes do not overlap. In an embodiment the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment a bispecific antibody molecule comprises a heavy chain variable region sequence and a light chain variable region sequence which have binding specificity for a first epitope and a heavy chain variable

region sequence and a light chain variable region sequence which have binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody having binding specificity for a first epitope and a half antibody having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a scFv or a Fab, or fragment thereof, have binding specificity for a first epitope and a scFv or a Fab, or fragment thereof, have binding specificity for a second epitope.

In an embodiment, an antibody molecule comprises a diabody, and a single-chain molecule, as well as an antigen-binding fragment of an antibody (*e.g.*, Fab, F(ab')₂, and Fv). For example, an antibody molecule can include a heavy (H) chain variable region sequence (abbreviated herein as VH), and a light (L) chain variable region sequence (abbreviated herein as VL). In an embodiment an antibody molecule comprises or consists of a heavy chain and a light chain (referred to herein as a half antibody). In another example, an antibody molecule includes two heavy (H) chain variable region sequences and two light (L) chain variable region sequence, thereby forming two antigen binding sites, such as Fab, Fab', F(ab')₂, Fc, Fd, Fd', Fv, single chain antibodies (scFv for example), single variable region antibodies, diabodies (Dab) (bivalent and bispecific), and chimeric (*e.g.*, humanized) antibodies, which may be produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. These functional antibody fragments retain the ability to selectively bind with their respective antigen or receptor. Antibodies and antibody fragments can be from any class of antibodies including, but not limited to, IgG, IgA, IgM, IgD, and IgE, and from any subclass (*e.g.*, IgG1, IgG2, IgG3, and IgG4) of antibodies. The a preparation of antibody molecules can be monoclonal or polyclonal. An antibody molecule can also be a human, humanized, CDR-grafted, or *in vitro* generated antibody. The antibody can have a heavy chain constant region chosen from, *e.g.*, IgG1, IgG2, IgG3, or IgG4. The antibody can also have a light chain chosen from, *e.g.*, kappa or lambda. The term “immunoglobulin” (Ig) is used interchangeably with the term “antibody” herein.

Examples of antigen-binding fragments of an antibody molecule include: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the

hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a diabody (dAb) fragment, which consists of a VH domain; (vi) a camelid or camelized variable region; (vii) a single chain Fv (scFv), *see e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883); (viii) a single domain antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

Antibody molecules include intact molecules as well as functional fragments thereof. Constant regions of the antibody molecules can be altered, *e.g.*, mutated, to modify the properties of the antibody (*e.g.*, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function).

Antibody molecules can also be single domain antibodies. Single domain antibodies can include antibodies whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain antibodies, antibodies naturally devoid of light chains, single domain antibodies derived from conventional 4-chain antibodies, engineered antibodies and single domain scaffolds other than those derived from antibodies. Single domain antibodies may be any of the art, or any future single domain antibodies. Single domain antibodies may be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, and bovine. According to another aspect of the invention, a single domain antibody is a naturally occurring single domain antibody known as heavy chain antibody devoid of light chains. Such single domain antibodies are disclosed in WO 9404678, for example. For clarity reasons, this variable region derived from a heavy chain antibody naturally devoid of light chain is known herein as a VHH or nanobody to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from antibodies raised in *Camelidae* species, for example in camel, llama, dromedary, alpaca and guanaco. Other species besides *Camelidae* may produce heavy chain antibodies naturally devoid of light chain; such VHHs are within the scope of the invention.

The VH and VL regions can be subdivided into regions of hypervariability, termed “complementarity determining regions” (CDR), interspersed with regions that are more conserved, termed “framework regions” (FR or FW).

The extent of the framework region and CDRs has been precisely defined by a number of methods (*see*, Kabat, E. A., *et al.* (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Chothia, C. *et al.* (1987) *J. Mol. Biol.* 196:901-917; and the AbM definition used by Oxford Molecular's AbM antibody modeling software. *See*, generally, *e.g.*, *Protein Sequence and Structure Analysis of Antibody Variable Domains*. In: *Antibody Engineering Lab Manual* (Ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg).

The terms “complementarity determining region,” and “CDR,” as used herein refer to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. In general, there are three CDRs in each heavy chain variable region (HCDR1, HCDR2, HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, LCDR3).

The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of known schemes, including those described by Kabat *et al.* (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (“Kabat” numbering scheme), Al-Lazikani *et al.*, (1997) *JMB* 273,927-948 (“Chothia” numbering scheme). As used herein, the CDRs defined according the “Chothia” number scheme are also sometimes referred to as “hypervariable loops.”

For example, under Kabat, the CDR amino acid residues in the heavy chain variable region (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain variable region (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under Chothia, the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the amino acid residues in VL are numbered 26-32 (LCDR1), 50-52 (LCDR2), and 91-96 (LCDR3).

Each VH and VL typically includes three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The antibody molecule can be a polyclonal or a monoclonal antibody.

The terms “monoclonal antibody” or “monoclonal antibody composition” as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

A monoclonal antibody can be made by hybridoma technology or by methods that do not use hybridoma technology (*e.g.*, recombinant methods).

The antibody can be recombinantly produced, *e.g.*, produced by phage display or by combinatorial methods.

Phage display and combinatorial methods for generating antibodies are known in the art (as described in, *e.g.*, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* International Publication No. WO 92/18619; Dower *et al.* International Publication No. WO 91/17271; Winter *et al.* International Publication WO 92/20791; Markland *et al.* International Publication No. WO 92/15679; Breitling *et al.* International Publication WO 93/01288; McCafferty *et al.* International Publication No. WO 92/01047; Garrard *et al.* International Publication No. WO 92/09690; Ladner *et al.* International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982, the contents of all of which are incorporated by reference herein).

In one embodiment, the antibody is a fully human antibody (*e.g.*, an antibody made in a mouse which has been genetically engineered to produce an antibody from a human immunoglobulin sequence), or a non-human antibody, *e.g.*, a rodent (mouse or rat), goat, primate (*e.g.*, monkey), camel antibody. Preferably, the non-human antibody is a rodent (mouse or rat antibody). Methods of producing rodent antibodies are known in the art.

Human monoclonal antibodies can be generated using transgenic mice carrying the human immunoglobulin genes rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein (see, *e.g.*, Wood *et al.* International Application WO 91/00906, Kucherlapati *et al.* PCT publication WO 91/10741; Lonberg *et al.* International Application WO 92/03918; Kay *et al.* International Application 92/03917; Lonberg, N. *et al.* 1994 *Nature* 368:856-859; Green, L.L. *et al.* 1994 *Nature Genet.* 7:13-21; Morrison, S.L. *et al.* 1994 *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Bruggeman *et al.*

1993 *Year Immunol* 7:33-40; Tuailon *et al.* 1993 *PNAS* 90:3720-3724; Bruggeman *et al.* 1991 *Eur J Immunol* 21:1323-1326).

An antibody molecule can be one in which the variable region, or a portion thereof, *e.g.*, the CDRs, are generated in a non-human organism, *e.g.*, a rat or mouse. Chimeric, CDR-grafted, and humanized antibodies are within the invention. Antibody molecules generated in a non-human organism, *e.g.*, a rat or mouse, and then modified, *e.g.*, in the variable framework or constant region, to decrease antigenicity in a human are within the invention.

An “effectively human” protein is a protein that does substantially not evoke a neutralizing antibody response, *e.g.*, the human anti-murine antibody (HAMA) response. HAMA can be problematic in a number of circumstances, *e.g.*, if the antibody molecule is administered repeatedly, *e.g.*, in treatment of a chronic or recurrent disease condition. A HAMA response can make repeated antibody administration potentially ineffective because of an increased antibody clearance from the serum (*see, e.g.*, Saleh *et al.*, *Cancer Immunol. Immunother.*, 32:180-190 (1990)) and also because of potential allergic reactions (*see, e.g.*, LoBuglio *et al.*, *Hybridoma*, 5:5117-5123 (1986)).

Chimeric antibodies can be produced by recombinant DNA techniques known in the art (see Robinson *et al.*, International Patent Publication PCT/US86/02269; Akira, *et al.*, European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, International Application WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988 *Science* 240:1041-1043); Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.*, 1987, *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.*, 1987, *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.*, 1988, *J. Natl Cancer Inst.* 80:1553-1559).

A humanized or CDR-grafted antibody will have at least one or two but generally all three recipient CDRs (of heavy and or light immunoglobulin chains) replaced with a donor CDR. The antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding to the antigen. Preferably, the donor will be a rodent antibody, *e.g.*, a rat or mouse antibody, and the recipient will be a human framework or a human consensus framework. Typically, the immunoglobulin providing the CDRs is called the “donor” and the

immunoglobulin providing the framework is called the “acceptor.” In one embodiment, the donor immunoglobulin is a non-human (*e.g.*, rodent). The acceptor framework is a naturally-occurring (*e.g.*, a human) framework or a consensus framework, or a sequence about 85% or higher, preferably 90%, 95%, 99% or higher identical thereto.

As used herein, the term “consensus sequence” refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related sequences (See *e.g.*, Winnaker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of proteins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence. A “consensus framework” refers to the framework region in the consensus immunoglobulin sequence.

An antibody molecule can be humanized by methods known in the art (*see e.g.*, Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi *et al.*, 1986, *BioTechniques* 4:214, and by Queen *et al.* US 5,585,089, US 5,693,761 and US 5,693,762, the contents of all of which are hereby incorporated by reference).

Humanized or CDR-grafted antibody molecules can be produced by CDR-grafting or CDR substitution, wherein one, two, or all CDRs of an immunoglobulin chain can be replaced. *See e.g.*, U.S. Patent 5,225,539; Jones *et al.* 1986 *Nature* 321:552-525; Verhoeyan *et al.* 1988 *Science* 239:1534; Beidler *et al.* 1988 *J. Immunol.* 141:4053-4060; Winter US 5,225,539, the contents of all of which are hereby expressly incorporated by reference. Winter describes a CDR-grafting method which may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987; Winter US 5,225,539), the contents of which is expressly incorporated by reference.

Also within the scope of the invention are humanized antibody molecules in which specific amino acids have been substituted, deleted or added. Criteria for selecting amino acids from the donor are described in US 5,585,089, *e.g.*, columns 12-16 of US 5,585,089, *e.g.*, columns 12-16 of US 5,585,089, the contents of which are hereby incorporated by reference. Other techniques for humanizing antibodies are described in Padlan *et al.* EP 519596 A1, published on December 23, 1992.

The antibody molecule can be a single chain antibody. A single-chain antibody (scFV) may be engineered (*see, for example, Colcher, D. et al. (1999) Ann N Y Acad Sci* 880:263-80;

and Reiter, Y. (1996) *Clin Cancer Res* 2:245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target protein.

In yet other embodiments, the antibody molecule has a heavy chain constant region chosen from, *e.g.*, the heavy chain constant regions of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE; particularly, chosen from, *e.g.*, the (*e.g.*, human) heavy chain constant regions of IgG1, IgG2, IgG3, and IgG4. In another embodiment, the antibody molecule has a light chain constant region chosen from, *e.g.*, the (*e.g.*, human) light chain constant regions of kappa or lambda. The constant region can be altered, *e.g.*, mutated, to modify the properties of the antibody (*e.g.*, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, and/or complement function). In one embodiment the antibody has: effector function; and can fix complement. In other embodiments the antibody does not; recruit effector cells; or fix complement. In another embodiment, the antibody has reduced or no ability to bind an Fc receptor. For example, it is a isotype or subtype, fragment or other mutant, which does not support binding to an Fc receptor, *e.g.*, it has a mutagenized or deleted Fc receptor binding region.

Methods for altering an antibody constant region are known in the art. Antibodies with altered function, *e.g.* altered affinity for an effector ligand, such as FcR on a cell, or the C1 component of complement can be produced by replacing at least one amino acid residue in the constant portion of the antibody with a different residue (*see e.g.*, EP 388,151 A1, U.S. Pat. No. 5,624,821 and U.S. Pat. No. 5,648,260, the contents of all of which are hereby incorporated by reference). Similar type of alterations could be described which if applied to the murine, or other species immunoglobulin would reduce or eliminate these functions.

An antibody molecule can be derivatized or linked to another functional molecule (*e.g.*, another peptide or protein). As used herein, a “derivatized” antibody molecule is one that has been modified. Methods of derivatization include but are not limited to the addition of a fluorescent moiety, a radionucleotide, a toxin, an enzyme or an affinity ligand such as biotin. Accordingly, the antibody molecules of the invention are intended to include derivatized and otherwise modified forms of the antibodies described herein, including immunoadhesion molecules. For example, an antibody molecule can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities,

such as another antibody (*e.g.*, a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

One type of derivatized antibody molecule is produced by crosslinking two or more antibodies (of the same type or of different types, *e.g.*, to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (*e.g.*, *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester) or homobifunctional (*e.g.*, disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill.

Multispecific antibody molecules

In embodiments, multispecific antibody molecules can comprise more than one antigen-binding site, where different sites are specific for different antigens. In embodiments, multispecific antibody molecules can bind more than one (*e.g.*, two or more) epitopes on the same antigen. In embodiments, multispecific antibody molecules comprise an antigen-binding site specific for a target cell (*e.g.*, cancer cell) and a different antigen-binding site specific for an immune effector cell. In one embodiment, the multispecific antibody molecule is a bispecific antibody molecule. Bispecific antibody molecules can be classified into five different structural groups: (i) bispecific immunoglobulin G (BsIgG); (ii) IgG appended with an additional antigen-binding moiety; (iii) bispecific antibody fragments; (iv) bispecific fusion proteins; and (v) bispecific antibody conjugates.

BsIgG is a format that is monovalent for each antigen. Exemplary BsIgG formats include but are not limited to crossMab, DAF (two-in-one), DAF (four-in-one), DutaMab, DT-IgG, knobs-in-holes common LC, knobs-in-holes assembly, charge pair, Fab-arm exchange, SEEDbody, triomab, LUZ-Y, Fcab, $\kappa\lambda$ -body, orthogonal Fab. *See* Spiess et al. Mol. Immunol. 67(2015):95-106. Exemplary BsIgGs include catumaxomab (Fresenius Biotech, Trion Pharma, Neopharm), which contains an anti-CD3 arm and an anti-EpCAM arm; and ertumaxomab (Neovii Biotech, Fresenius Biotech), which targets CD3 and HER2. In some embodiments, BsIgG comprises heavy chains that are engineered for heterodimerization. For example, heavy chains can be engineered for heterodimerization using a “knobs-into-holes” strategy, a SEED

platform, a common heavy chain (e.g., in $\kappa\lambda$ -bodies), and use of heterodimeric Fc regions. *See* Spiess et al. Mol. Immunol. 67(2015):95-106. Strategies that have been used to avoid heavy chain pairing of homodimers in BsIgG include knobs-in-holes, duobody, azymetric, charge pair, HA-TF, SEEDbody, and differential protein A affinity. *See Id.* BsIgG can be produced by separate expression of the component antibodies in different host cells and subsequent purification/assembly into a BsIgG. BsIgG can also be produced by expression of the component antibodies in a single host cell. BsIgG can be purified using affinity chromatography, e.g., using protein A and sequential pH elution.

IgG appended with an additional antigen-binding moiety is another format of bispecific antibody molecules. For example, monospecific IgG can be engineered to have bispecificity by appending an additional antigen-binding unit onto the monospecific IgG, e.g., at the N- or C-terminus of either the heavy or light chain. Exemplary additional antigen-binding units include single domain antibodies (e.g., variable heavy chain or variable light chain), engineered protein scaffolds, and paired antibody variable regions (e.g., single chain variable fragments or variable fragments). *See Id.* Examples of appended IgG formats include dual variable domain IgG (DVD-Ig), IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, zyboby, and DVI-IgG (four-in-one). *See* Spiess et al. Mol. Immunol. 67(2015):95-106. An example of an IgG-scFv is MM-141 (Merrimack Pharmaceuticals), which binds IGF-1R and HER3. Examples of DVD-Ig include ABT-981 (AbbVie), which binds IL-1 α and IL-1 β ; and ABT-122 (AbbVie), which binds TNF and IL-17A.

Bispecific antibody fragments (BsAb) are a format of bispecific antibody molecules that lack some or all of the antibody constant domains. For example, some BsAb lack an Fc region. In embodiments, bispecific antibody fragments include heavy and light chain regions that are connected by a peptide linker that permits efficient expression of the BsAb in a single host cell. Exemplary bispecific antibody fragments include but are not limited to nanobody, nanobody-HAS, BiTE, Diabody, DART, TandAb, scDiabody, scDiabody-CH3, Diabody-CH3, triple body, miniantibody, minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv, scFv-CH-CL-scFv, F(ab')₂, F(ab')₂-scFv₂, scFv-KIH, Fab-scFv-Fc, tetravalent HCAb, scDiabody-Fc, Diabody-Fc, tandem scFv-Fc, and intrabody. *See Id.* For example, the BiTE format comprises tandem scFvs, where the component scFvs bind to CD3 on T cells and a surface antigen on cancer cells

Bispecific fusion proteins include antibody fragments linked to other proteins, e.g., to add additional specificity and/or functionality. An example of a bispecific fusion protein is an immTAC, which comprises an anti-CD3 scFv linked to an affinity-matured T-cell receptor that recognizes HLA-presented peptides. In embodiments, the dock-and-lock (DNL) method can be used to generate bispecific antibody molecules with higher valency. Also, fusions to albumin binding proteins or human serum albumin can be extend the serum half-life of antibody fragments. *See Id.*

In embodiments, chemical conjugation, e.g., chemical conjugation of antibodies and/or antibody fragments, can be used to create BsAb molecules. *See Id.* An exemplary bispecific antibody conjugate includes the CovX-body format, in which a low molecular weight drug is conjugated site-specifically to a single reactive lysine in each Fab arm or an antibody or fragment thereof. In embodiments, the conjugation improves the serum half-life of the low molecular weight drug. An exemplary CovX-body is CVX-241 (NCT01004822), which comprises an antibody conjugated to two short peptides inhibiting either VEGF or Ang2. *See Id.*

The antibody molecules can be produced by recombinant expression, e.g., of at least one or more component, in a host system. Exemplary host systems include eukaryotic cells (e.g., mammalian cells, e.g., CHO cells, or insect cells, e.g., SF9 or S2 cells) and prokaryotic cells (e.g., *E. coli*). Bispecific antibody molecules can be produced by separate expression of the components in different host cells and subsequent purification/assembly. Alternatively, the antibody molecules can be produced by expression of the components in a single host cell. Purification of bispecific antibody molecules can be performed by various methods such as affinity chromatography, e.g., using protein A and sequential pH elution. In other embodiments, affinity tags can be used for purification, e.g., histidine-containing tag, myc tag, or streptavidin tag.

Multispecific antibody molecules targeting CSF1R

In one aspect, disclosed herein is a multispecific antibody molecule comprising a CSF1R binding moiety. In some embodiments, the CSF1R binding moiety comprises an anti-CSF1R antibody molecule. Exemplary anti-CSF1R antibody molecule sequences are described in WO2009026303A1; WO2011123381A1; WO2016207312A1; WO2016106180A1; US20160220669A1; US20160326254A1; WO2013169264A1; WO2013087699A1;

WO2011140249A2; WO2011131407A1; WO2011123381A1; WO2011107553A1; and WO2011070024A1, all of which are herein incorporated by reference in their entirety. In some embodiments, the CSF1R binding moiety comprises the CDR (e.g., one, two, three, four, five, or all six CDRs), VH, VL, heavy chain, or light chain sequences of emactuzumab, or a sequence substantially identical thereto (e.g., at least 95% identical thereto, e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions)). In some embodiments, the CSF1R binding moiety comprises the CDR (e.g., one, two, three, four, five, or all six CDRs), VH, VL, heavy chain, or light chain sequences of cabiralizumab, or a sequence substantially identical thereto (e.g., .., at least 95% identical thereto, e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions)). In some embodiments, the CSF1R binding moiety comprises the CDR (e.g., one, two, three, four, five, or all six CDRs), VH, VL, heavy chain, or light chain sequences of AMG820, or a sequence substantially identical thereto (e.g., .., at least 95% identical thereto, e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions)). In some embodiments, the CSF1R binding moiety comprises the CDR (e.g., one, two, three, four, five, or all six CDRs), VH, VL, heavy chain, or light chain sequences of IMC-CS4, or a sequence substantially identical thereto (e.g., .., at least 95% identical thereto, e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions)). In some embodiments, the CSF1R binding moiety comprises a VH or VL amino acid sequence disclosed in Table 8, a CDR of a VH or VL amino acid sequence disclosed in Table 8, or a sequence substantially identical thereto.

Table 8. Exemplary anti-CSF1R antibody molecule sequences

SEQ ID NO	Description	Sequence
SEQ ID NO: 48	α mCSF1R VH	QVQLQQSGAELVKPGSSVKISCKASGYTFTSNFMHWIKQQPGNGLE WIGWIYPGDGDTEYNQKFNGKATLTADKSSSTAYMQLSSLTSEDSA VYFCAVNYGGYVLDAWGQGASVTVSS
SEQ ID NO: 50	α mCSF1R VL	EIVLTQSPPTMAASPGEKVTITCRASSSTNYMSWYQQKSGASPKPWI YETSKLASGVDPDRFSGSGSGTSYSFTISSMETEDAATYYCHQWSSTP

		LTFGSGTKLEIK
SEQ ID NO: 66	α hCSF1R emactuzumab VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDISWVRQAPGQGL EWMGVIWTDGGTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSD TAVYYCARDQRLYFDVWGQGTTVTVSS
SEQ ID NO: 67	α hCSF1R emactuzumab VL	DIQMTQSPSSLSASVGDRVTITCRASEDVNTYVSWYQQKPGKAPKL LIYAASNRYTGVPSPRFSGSGSGTDFTLTISLQPEDFATYYCQQSF SYPTFGQGTKLEIK
SEQ ID NO: 69	α hCSF1R cabiralizumab VH	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDNYMIWVRQAPGQGL EWMGDINPYNGGTTFNQKFKGRVTITADKSTSTAYMELSSLRSED TAVYYCARESPYFSNLYVMDYWGQGTLLTVSS
SEQ ID NO: 70	α hCSF1R cabiralizumab VL	EIVLTQSPATLSLSPGERATLSCKASQSVDDYDGDNYMNWYQQKPG QAPRLLIYAASNLESGIPARFSGSGSGTDFTLTISLEPEDFAVYYCH LSNEDLSTFGGGTKVEIK

Multispecific antibody molecules targeting CCR2

In one aspect, disclosed herein is a multispecific antibody molecule comprising a CCR2 binding moiety. Exemplary CCR2 antibodies are described herein as well as in WO2013192596A2; WO2010021697A2; WO2001057226A1; and WO1997031949A1, all of which are herein incorporated by reference in their entirety. In some embodiments, the CCR2 binding moiety comprises the CDR (e.g., one, two, three, four, five, or all six CDRs), VH, VL, heavy chain, or light chain sequences of ploxalizumab, or a sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions)). In some embodiments, the CCR2 binding moiety comprises a VH or VL amino acid sequence disclosed in Table 9, a CDR of a VH or VL amino acid sequence disclosed in Table 9, or a sequence substantially identical thereto.

Table 9. Exemplary anti-CCR2 antibody molecule sequences

SEQ ID NO	Description	Sequence
SEQ ID NO: 44	α CCR2 MC12 VH	QVQLQESGPGLVQPSQTLSTCTVSGFSLTDFSVHWVRQPPGKGLE WMGRIRSEGNTDYNALKSRLSISRDTSKSQVFLKMNSLQTEDTAIY FCTRGDILGFGYWGQGVMTVSS
SEQ ID NO: 45	α CCR2 MC12 VL	DIVMTQSPLSVSVTPGESASISCRSSKSLHFKGITFVYWYLQKPGQS PQLLIFRMSSLASGVPDRFSGSGSETDFTLKISRVEAEDVGTYYCGQ LLENPYTFGAGTKLELK
SEQ ID NO: 54	α hCCR2 ploxalizumab VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMNWVRQAPGKGL EWVGRIRTKNNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLKT EDTAVYYCTTFYGNQVWGQGTLLTVSS
SEQ ID NO: 57	α hCCR2 ploxalizumab	DVVMTQSPLSLPVTLGQPASISCKSSQSLLDSDGKTFLNWFQQRPGQ SPRRLIYLVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCW

	VL	QGTHFPYTFGQGTRLEIK
SEQ ID NO: 59	α hCCR2 D1 VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTGYHMHWRQAPGQG LEWMGWINPNSGVTKYAQKFQGRVTMTRDTSINTAYMELSRRLRFD DTDVYYCATGGFGYWGEGLVTVSS
SEQ ID NO: 60	α hCCR2 D1 VL	LPVLTQPPSVSKGLRQTATLTCTGNSNNVGNQGAAWLQQHQGQPP KLLSYRNHNRPSPGVSEFSPSRSGDTSSLTITGLQPEDEADYYCLAW DSSLRAFVFGTGTKLTVL
SEQ ID NO: 62	α hCCR2 42G7 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFSSYYMHWRQAPGQG LEWMGIINPSGGNTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSED TAVYYCARGGYQLPHGRARAFDMWGQGTMTVTSS
SEQ ID NO: 63	α hCCR2 42G7 VL	AIRMTQSPLSLPVTLGQPASISCTSSQSLVYRDGTTYLNWFQQRPGQ SPRRLIYKVSNRDSGVDPDRFTGSGSGTTFTLTISRVEADVGIYYCM QGTHWPLTFGQGTKVEIK
SEQ ID NO: 64	α hCCR2 43G12 VH	EVQLVESGGGLVQPGGSLRLSCVASGFTFSDYWMSWVRQAPGKGL EWVANIKKDGSVNYYVDSVKGRFTISRDNANKNSLYLQMNSLRAED TAVYYCTRFDYWGQGTTLTVSS
SEQ ID NO: 65	α hCCR2 43G12 VL	QAGLTQPPSVSKGLRQTATLTCTGNSNNVGNQGAAWLQQHQGHPP KLLFYRNNNRASGISERLSASRSGNTASLTITGLQPEDEADYYCLTW DSSLSVVVFGGGTKLTVL

Multispecific antibody molecules targeting PD-L1

In one aspect, disclosed herein is a multispecific antibody molecule comprising a PD-L1 binding moiety. In some embodiments, the PD-L1 binding moiety comprises an anti-PD-L1 antibody molecule. Exemplary anti-PD-L1 antibody molecule sequences are described in WO2013079174, WO 2010077634, WO2007/005874, and US20120039906, all of which are herein incorporated by reference in their entirety. In some embodiments, the PD-L1 binding moiety comprises the CDR (e.g., one, two, three, four, five, or all six CDRs), VH, VL, heavy chain, or light chain sequences of durvalumab, or a sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions)). In some embodiments, the PD-L1 binding moiety comprises the CDR (e.g., one, two, three, four, five, or all six CDRs), VH, VL, heavy chain, or light chain sequences of atezolizumab, or a sequence substantially identical thereto (e.g., at least 95% identical thereto, e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions)). In some embodiments, the PD-L1 binding moiety comprises the CDR (e.g., one, two, three, four, five, or all six CDRs), VH, VL, heavy chain, or light chain sequences of avelumab, or a sequence substantially identical thereto (e.g., at least 95% identical thereto, e.g.,

95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions)). In some embodiments, the PD-L1 binding moiety comprises a VH or VL amino acid sequence disclosed in Table 10, a CDR of a VH or VL amino acid sequence disclosed in Table 10, or a sequence substantially identical thereto.

Table 10. Exemplary anti-PD-L1 antibody molecule sequences

SEQ ID NO	Description	Sequence
SEQ ID NO: 109	α PD-L1 durvalumab VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGL EWVANIKQDGSEKYYVDSVKGRFTISRDNKNSLYLQMNSLRAED TAVYYCAREGGWFGELAFDYWGQGTLVTVSS
SEQ ID NO: 110	α PD-L1 durvalumab VL	EIVLTQSPGTLSSLSPGERATLSCRASQRVSSSYLAWYQQKPGQAPRL LIYDASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSL PWTFGGQGTKVEIK
SEQ ID NO: 111	α PD-L1 atezolizumab VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLE WVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDT AVYYCARRHWPGGFDYWGQGTLVTVSS
SEQ ID NO: 112	α PD-L1 atezolizumab VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKL LIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYLYH PATFGQGGTKVEIK
SEQ ID NO: 113	α PD-L1 avelumab VH	EVQLLES GGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLE WVSSIYPSGGITFYADTVKGRFTISRDNKNTLYLQMNSLRAEDTAV YYCARIKLGTVTTVDYWGQGTLVTVSS
SEQ ID NO: 114	α PD-L1 avelumab VL	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAP KLMIYDVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSY TSSSTRVFGTGTKVTVL

CDR-grafted scaffolds

In embodiments, the antibody molecule is a CDR-grafted scaffold domain. In embodiments, the scaffold domain is based on a fibronectin domain, e.g., fibronectin type III domain. The overall fold of the fibronectin type III (Fn3) domain is closely related to that of the smallest functional antibody fragment, the variable region of the antibody heavy chain. There are three loops at the end of Fn3; the positions of BC, DE and FG loops approximately correspond to those of CDR1, 2 and 3 of the VH domain of an antibody. Fn3 does not have disulfide bonds; and therefore Fn3 is stable under reducing conditions, unlike antibodies and their fragments (see, e.g., WO 98/56915; WO 01/64942; WO 00/34784). An Fn3 domain can be modified (e.g., using CDRs or hypervariable loops described herein) or varied, e.g., to select domains that bind to an antigen/marker/cell described herein.

In embodiments, a scaffold domain, e.g., a folded domain, is based on an antibody, e.g., a “minibody” scaffold created by deleting three beta strands from a heavy chain variable region of a monoclonal antibody (see, e.g., Tramontano et al., 1994, J Mol. Recognit. 7:9; and Martin et al., 1994, EMBO J. 13:5303-5309). The “minibody” can be used to present two hypervariable loops. In embodiments, the scaffold domain is a V-like domain (see, e.g., Coia et al. WO 99/45110) or a domain derived from tendamistatin, which is a 74 residue, six-strand beta sheet sandwich held together by two disulfide bonds (see, e.g., McConnell and Hoess, 1995, J Mol. Biol. 250:460). For example, the loops of tendamistatin can be modified (e.g., using CDRs or hypervariable loops) or varied, e.g., to select domains that bind to a marker/antigen/cell described herein. Another exemplary scaffold domain is a beta-sandwich structure derived from the extracellular domain of CTLA-4 (see, e.g., WO 00/60070).

Other exemplary scaffold domains include but are not limited to T-cell receptors; MHC proteins; extracellular domains (e.g., fibronectin Type III repeats, EGF repeats); protease inhibitors (e.g., Kunitz domains, ecotin, BPTI, and so forth); TPR repeats; trifoil structures; zinc finger domains; DNA-binding proteins; particularly monomeric DNA binding proteins; RNA binding proteins; enzymes, e.g., proteases (particularly inactivated proteases), RNase; chaperones, e.g., thioredoxin, and heat shock proteins; and intracellular signaling domains (such as SH2 and SH3 domains). See, e.g., US 20040009530 and US 7,501,121, incorporated herein by reference.

In embodiments, a scaffold domain is evaluated and chosen, e.g., by one or more of the following criteria: (1) amino acid sequence, (2) sequences of several homologous domains, (3) 3-dimensional structure, and/or (4) stability data over a range of pH, temperature, salinity, organic solvent, oxidant concentration. In embodiments, the scaffold domain is a small, stable protein domain, e.g., a protein of less than 100, 70, 50, 40 or 30 amino acids. The domain may include one or more disulfide bonds or may chelate a metal, e.g., zinc.

Exemplary structures of the multifunctional molecules defined herein are described below. Exemplary structures are further described in: Weidle U et al. (2013) The Intriguing Options of Multispecific Antibody Formats for Treatment of Cancer. *Cancer Genomics & Proteomics* 10: 1-18 (2013); and Spiess C et al. (2015) Alternative molecular formats and

therapeutic applications for bispecific antibodies. *Molecular Immunology* 67: 95-106; the full contents of each of which is incorporated by reference herein).

Heterodimerized Antibody Molecules

Heterodimerized bispecific antibodies are based on the natural IgG structure, wherein the two binding arms recognize different antigens. IgG derived formats that enable defined monovalent (and simultaneous) antigen binding are generated by forced heavy chain heterodimerization, combined with technologies that minimize light chain mispairing (e.g., common light chain). Forced heavy chain heterodimerization can be obtained using, e.g., knob-in-hole OR strand exchange engineered domains (SEED).

Knob-in-Hole

Knob-in-Hole as described in US 5,731,116, US 7,476,724 and Ridgway, J. *et al.* (1996) *Prot. Engineering* 9(7): 617-621, broadly involves: (1) mutating the CH3 domain of one or both antibodies to promote heterodimerization; and (2) combining the mutated antibodies under conditions that promote heterodimerization. “Knobs” or “protuberances” are typically created by replacing a small amino acid in a parental antibody with a larger amino acid (e.g., T366Y or T366W); “Holes” or “cavities” are created by replacing a larger residue in a parental antibody with a smaller amino acid (e.g., Y407T, T366S, L368A and/or Y407V), numbered based on the Eu numbering system.

Strand Exchange Engineered Domains (SEED)

SEED is based on sequence exchanges between IgG1 and IgA to create non-identical chains which heterodimerize preferentially. Alternating sequences from human IgA and IgG in the SEED CH3 domains generate two asymmetric but complementary domains, designated AG and GA. The SEED design allows efficient generation of AG/GA heterodimers, while disfavoring homodimerization of AG and GA SEED CH3 domains.

Common Light Chain & CrossMab

Light chain mispairing must be avoided to generate homogenous preparations of bispecific IgGs. One way to achieve this is through the use of the common light chain principle,

i.e. combining two binders that share one light chain but still have separate specificities. Another option is the CrossMab technology which avoids non-specific L chain mispairing by exchanging CH1 and CL domains in the Fab of one half of the bispecific antibody. Such crossover variants retain binding specificity and affinity, but make the two arms so different that L chain mispairing is prevented.

Antibody-Based Fusions

A variety of formats can be generated which contain additional binding entities attached to the N or C terminus of antibodies. These fusions with single chain or disulfide stabilized Fvs or Fabs result in the generation of tetravalent molecules with bivalent binding specificity for each antigen. Combinations of scFvs and scFabs with IgGs enable the production of molecules which can recognize three or more different antigens.

Antibody-Fab Fusion

Antibody-Fab fusions are bispecific antibodies comprising a traditional antibody to a first target and a Fab to a second target fused to the C terminus of the antibody heavy chain. Commonly the antibody and the Fab will have a common light chain. Antibody fusions can be produced by (1) engineering the DNA sequence of the target fusion, and (2) transfecting the target DNA into a suitable host cell to express the fusion protein. It seems like the antibody-scFv fusion may be linked by a (Gly)-Ser linker between the C-terminus of the CH3 domain and the N-terminus of the scFv, as described by Coloma, J. *et al.* (1997) *Nature Biotech* 15:159.

Antibody-scFv Fusion

Antibody-scFv Fusions are bispecific antibodies comprising a traditional antibody and a scFv of unique specificity fused to the C terminus of the antibody heavy chain. The scFv can be fused to the C terminus through the Heavy Chain of the scFv either directly or through a linker peptide. Antibody fusions can be produced by (1) engineering the DNA sequence of the target fusion, and (2) transfecting the target DNA into a suitable host cell to express the fusion protein. It seems like the antibody-scFv fusion may be linked by a (Gly)-Ser linker between the C-terminus of the CH3 domain and the N-terminus of the scFv, as described by Coloma, J. *et al.* (1997) *Nature Biotech* 15:159.

Variable Domain Immunoglobulin DVD

A related format is the dual variable domain immunoglobulin (DVD), which are composed of VH and VL domains of a second specificity placed upon the N termini of the V domains by shorter linker sequences.

Fc-containing entities (mini-antibodies)

Fc-containing entities, also known as mini-antibodies, can be generated by fusing scFv to the C-termini of constant heavy region domain 3 (CH3-scFv) and/or to the hinge region (scFv-hinge-Fc) of an antibody with a different specificity. Trivalent entities can also be made which have disulfide stabilized variable regions (without peptide linker) fused to the C-terminus of CH3 domains of IgGs.

Fc-less Bispecifics

Fc-less bispecifics are characterized by generally having smaller size than Fc-containing entities. Common bispecifics of this class include Fab-scFv2 and Fab-scFv molecules. This class also includes, e.g., BiTEs (bispecific T-cell engagers), diabodies, TandAbs (tetravalent tandem antibodies), and DARTs (dual affinity retargeting molecules). BiTEs are created by fusing two scFvs via a flexible linker peptide. Diabodies consist of two VH and two VL domains from two different antibodies. Interaction only with complementary domains on another chain is achieved by attaching domains with short linker peptides which permits pairing only with VH and VL domains. VH of the first binder linked to the VL of the second binder is co-expressed with the VH of the second antibody linked to VL of the first antibody. TandAbs molecules are generated by functional dimerization of a protein consisting of four antibody variable H- and L-chains in an orientation that prevents intramolecular pairing. DARTs are entities that are stabilized by disulfide bonds which apply a similar design concept to that of diabodies.

Kappa/Lambda Formats

Multispecific molecules (e.g., multispecific antibody molecules) that include the lambda light chain polypeptide and a kappa light chain polypeptides, can be used to allow for heterodimerization. Methods for generating bispecific antibody molecules comprising the

lambda light chain polypeptide and a kappa light chain polypeptides are disclosed in PCT/US2017/53053 filed on September 22, 2017, incorporated herein by reference in its entirety.

In embodiments, the multispecific molecules include a multispecific antibody molecule, e.g., an antibody molecule comprising two binding specificities, e.g., a bispecific antibody molecule. The multispecific antibody molecule includes:

- a lambda light chain polypeptide 1 (LLCP1) specific for a first epitope;
- a heavy chain polypeptide 1 (HCP1) specific for the first epitope;
- a kappa light chain polypeptide 2 (KLCP2) specific for a second epitope; and
- a heavy chain polypeptide 2 (HCP2) specific for the second epitope.

“Lambda light chain polypeptide 1 (LLCP1)”, as that term is used herein, refers to a polypeptide comprising sufficient light chain (LC) sequence, such that when combined with a cognate heavy chain variable region, can mediate specific binding to its epitope and complex with an HCP1. In an embodiment it comprises all or a fragment of a CH1 region. In an embodiment, an LLCP1 comprises LC-CDR1, LC-CDR2, LC-CDR3, FR1, FR2, FR3, FR4, and CH1, or sufficient sequence therefrom to mediate specific binding of its epitope and complex with an HCP1. LLCP1, together with its HCP1, provide specificity for a first epitope (while KLCP2, together with its HCP2, provide specificity for a second epitope). As described elsewhere herein, LLCP1 has a higher affinity for HCP1 than for HCP2.

“Kappa light chain polypeptide 2 (KLCP2)”, as that term is used herein, refers to a polypeptide comprising sufficient light chain (LC) sequence, such that when combined with a cognate heavy chain variable region, can mediate specific binding to its epitope and complex with an HCP2. In an embodiment it comprises all or a fragment of a CH1 region. In an embodiment, a KLCP2 comprises LC-CDR1, LC-CDR2, LC-CDR3, FR1, FR2, FR3, FR4, and CH1, or sufficient sequence therefrom to mediate specific binding of its epitope and complex with an HCP2. KLCP2, together with its HCP2, provide specificity for a second epitope (while LLCP1, together with its HCP1, provide specificity for a first epitope).

“Heavy chain polypeptide 1 (HCP1)”, as that term is used herein, refers to a polypeptide comprising sufficient heavy chain (HC) sequence, e.g., HC variable region sequence, such that when combined with a cognate LLCP1, can mediate specific binding to its epitope and complex

with an HCP1. In an embodiment it comprises all or a fragment of a CH1 region. In an embodiment, it comprises all or a fragment of a CH2 and/or CH3 region. In an embodiment an HCP1 comprises HC-CDR1, HC-CDR2, HC-CDR3, FR1, FR2, FR3, FR4, CH1, CH2, and CH3, or sufficient sequence therefrom to: (i) mediate specific binding of its epitope and complex with an LLC1, (ii) to complex preferentially, as described herein to LLC1 as opposed to KLC2; and (iii) to complex preferentially, as described herein, to an HCP2, as opposed to another molecule of HCP1. HCP1, together with its LLC1, provide specificity for a first epitope (while KLC2, together with its HCP2, provide specificity for a second epitope).

“Heavy chain polypeptide 2 (HCP2)”, as that term is used herein, refers to a polypeptide comprising sufficient heavy chain (HC) sequence, e.g., HC variable region sequence, such that when combined with a cognate LLC1, can mediate specific binding to its epitope and complex with an HCP1. In an embodiment it comprises all or a fragment of a CH1 region. In an embodiment it comprises all or a fragment of a CH2 and/or CH3 region. In an embodiment an HCP1 comprises HC-CDR1, HC-CDR2, HC-CDR3, FR1, FR2, FR3, FR4, CH1, CH2, and CH3, or sufficient sequence therefrom to: (i) mediate specific binding of its epitope and complex with an KLC2, (ii) to complex preferentially, as described herein to KLC2 as opposed to LLC1; and (iii) to complex preferentially, as described herein, to an HCP1, as opposed to another molecule of HCP2. HCP2, together with its KLC2, provide specificity for a second epitope (while LLC1, together with its HCP1, provide specificity for a first epitope).

In some embodiments of the multispecific antibody molecule disclosed herein:

LLC1 has a higher affinity for HCP1 than for HCP2; and/or

KLC2 has a higher affinity for HCP2 than for HCP1.

In embodiments, the affinity of LLC1 for HCP1 is sufficiently greater than its affinity for HCP2, such that under preselected conditions, e.g., in aqueous buffer, e.g., at pH 7, in saline, e.g., at pH 7, or under physiological conditions, at least 75%, 80, 90, 95, 98, 99, 99.5, or 99.9 % of the multispecific antibody molecule molecules have a LLC1 complexed, or interfaced with, a HCP1.

In some embodiments of the multispecific antibody molecule disclosed herein:

the HCP1 has a greater affinity for HCP2, than for a second molecule of HCP1; and/or

the HCP2 has a greater affinity for HCP1, than for a second molecule of HCP2.

In embodiments, the affinity of HCP1 for HCP2 is sufficiently greater than its affinity for a second molecule of HCP1, such that under preselected conditions, e.g., in aqueous buffer, e.g., at pH 7, in saline, e.g., at pH 7, or under physiological conditions, at least 75%, 80, 90, 95, 98, 99 99.5 or 99.9 % of the multispecific antibody molecule molecules have a HCP1 complexed, or interfaced with, a HCP2.

In another aspect, disclosed herein is a method for making, or producing, a multispecific antibody molecule. The method includes:

(i) providing a first heavy chain polypeptide (e.g., a heavy chain polypeptide comprising one, two, three or all of a first heavy chain variable region (first VH), a first CH1, a first heavy chain constant region (e.g., a first CH2, a first CH3, or both));

(ii) providing a second heavy chain polypeptide (e.g., a heavy chain polypeptide comprising one, two, three or all of a second heavy chain variable region (second VH), a second CH1, a second heavy chain constant region (e.g., a second CH2, a second CH3, or both));

(iii) providing a lambda chain polypeptide (e.g., a lambda light variable region (VL λ), a lambda light constant chain (VL λ), or both) that preferentially associates with the first heavy chain polypeptide (e.g., the first VH); and

(iv) providing a kappa chain polypeptide (e.g., a lambda light variable region (VL κ), a lambda light constant chain (VL κ), or both) that preferentially associates with the second heavy chain polypeptide (e.g., the second VH),

under conditions where (i)-(iv) associate.

In embodiments, the first and second heavy chain polypeptides form an Fc interface that enhances heterodimerization.

In embodiments, (i)-(iv) (e.g., nucleic acid encoding (i)-(iv)) are introduced in a single cell, e.g., a single mammalian cell, e.g., a CHO cell. In embodiments, (i)-(iv) are expressed in the cell.

In embodiments, (i)-(iv) (e.g., nucleic acid encoding (i)-(iv)) are introduced in different cells, e.g., different mammalian cells, e.g., two or more CHO cell. In embodiments, (i)-(iv) are expressed in the cells.

In one embodiment, the method further comprises purifying a cell-expressed antibody molecule, e.g., using a lambda- and/or- kappa-specific purification, e.g., affinity chromatography.

In embodiment, the method further comprises evaluating the cell-expressed multispecific antibody molecule. For example, the purified cell-expressed multispecific antibody molecule can be analyzed by techniques known in the art, include mass spectrometry. In one embodiment, the purified cell-expressed antibody molecule is cleaved, e.g., digested with papain to yield the Fab moieties and evaluated using mass spectrometry.

In embodiment, the method produces correctly paired kappa/lambda multispecific, e.g., bispecific, antibody molecules in a high yield, e.g., at least 75%, 80, 90, 95, 98, 99 99.5 or 99.9 %.

In other embodiment, the multispecific, e.g., a bispecific, antibody molecule that includes:

(i) a first heavy chain polypeptide (HCP1) (e.g., a heavy chain polypeptide comprising one, two, three or all of a first heavy chain variable region (first VH), a first CH1, a first heavy chain constant region (e.g., a first CH2, a first CH3, or both)), e.g., wherein the HCP1 binds to a first epitope;

(ii) a second heavy chain polypeptide (HCP2) (e.g., a heavy chain polypeptide comprising one, two, three or all of a second heavy chain variable region (second VH), a second CH1, a second heavy chain constant region (e.g., a second CH2, a second CH3, or both)), e.g., wherein the HCP2 binds to a second epitope;

(iii) a lambda light chain polypeptide (LLCP1) (e.g., a lambda light variable region (VLl), a lambda light constant chain (VLl), or both) that preferentially associates with the first heavy chain polypeptide (e.g., the first VH), e.g., wherein the LLCP1 binds to a first epitope; and

(iv) a kappa light chain polypeptide (KLCP2) (e.g., a lambda light variable region (VLk), a lambda light constant chain (VLk), or both) that preferentially associates with the second heavy chain polypeptide (e.g., the second VH), e.g., wherein the KLCP2 binds to a second epitope.

In embodiment, the first and second heavy chain polypeptides form an Fc interface that enhances heterodimerization. In embodiment, the multispecific antibody molecule has a first binding specificity that includes a hybrid VLl-CLl heterodimerized to a first heavy chain variable region connected to the Fc constant, CH2-CH3 domain (having a knob modification) and a

second binding specificity that includes a hybrid VLk-CLk heterodimerized to a second heavy chain variable region connected to the Fc constant, CH2-CH3 domain (having a hole modification).

Multispecific molecules comprising non-contiguous polypeptides

In one embodiment, the multispecific molecule is not a single polypeptide chain.

In one embodiment, the antibody molecule includes two, complete heavy chains and two, complete light chains. In one embodiment, the multispecific molecules having at least two or at least three non-contiguous polypeptide chains include a first and second heavy chain constant regions (e.g., a first and second Fc region) in at least two non-contiguous polypeptide chains, e.g., as described herein.

In embodiments, the multispecific molecule is a bispecific or bifunctional molecule, wherein the first and second polypeptides (i) and (ii) are non-contiguous, e.g., are two separate polypeptide chains. In some embodiments, the first and second polypeptides (i) and (ii) include a paired amino acid substitution at a position chosen from one or more of 347, 349, 350, 351, 366, 368, 370, 392, 394, 395, 397, 398, 399, 405, 407, or 409, e.g., of the Fc region of human IgG1, numbered based on the Eu numbering system. For example, the first heavy chain constant region (e.g., the first Fc region) can include an amino acid substitution chosen from: T366S, L368A, or Y407V (e.g., corresponding to a cavity or hole), and the second heavy chain constant region (e.g., the second Fc region) includes a T366W (e.g., corresponding to a protuberance or knob), numbered based on the Eu numbering system. In some embodiments, the first and second polypeptides are a first and second member of a heterodimeric first and second Fc region.

In some embodiments, the first polypeptide has the following configuration from N-to-C:

(a) a first portion of a first antigen domain, e.g., a first VH-CH1 of a Fab molecule, that binds to a first antigen, e.g., CSF1R, connected, optionally via a linker to, the first heavy chain constant region (e.g., the CH2 connected to the CH3 region) (e.g., a first Fc region); (b) a first portion of a second antigen domain, e.g., a second VH-CH1 of a Fab molecule, that binds to a second antigen, e.g., CCR2 or CXCR2, connected, optionally via a linker to, the second heavy chain constant region (e.g., the CH2 connected to the CH3 region) (e.g., a first Fc region); (c) the third polypeptide has the following configuration from N-to-C: a second portion of the first antigen domain, e.g., a first VL-CL of the Fab, where the VL is of kappa subtype and binds to

the first antigen, e.g., CSF1R (e.g., the same antigen bound by the first VH-CH1); (d) the fourth polypeptide has the following configuration from N-to-C: a second portion of the second antigen domain, e.g. a second VL-CL of the Fab, where the VL is of lambda subtype and binds to a second antigen, e.g., a cancer antigen, e.g., CCR2 or CXCR2 (e.g., the same antigen bound by the second VH-CH1).

In embodiments, the first heavy chain constant region (e.g., the first CH2-CH3 region) includes a protuberance or knob, e.g., as described herein. In embodiments, the second heavy chain constant region (e.g., the second CH2-CH3 region) includes a cavity or hole. In embodiments, the first and second heavy chain constant regions promote heterodimerization of the bispecific molecule.

Nucleic Acids

The invention also features nucleic acids comprising nucleotide sequences that encode heavy and light chain variable regions and CDRs or hypervariable loops of the antibody molecules, as described herein. For example, the invention features a first and second nucleic acid encoding heavy and light chain variable regions, respectively, of an antibody molecule chosen from one or more of the antibody molecules disclosed herein. The nucleic acid can comprise a nucleotide sequence as set forth in the tables herein, or a sequence substantially identical thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 3, 6, 15, 30, or 45 nucleotides from the sequences shown in the tables herein).

In certain embodiments, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a heavy chain variable region having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, *e.g.*, conserved substitutions). In other embodiments, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a light chain variable region having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, *e.g.*, conserved substitutions). In yet another embodiment, the nucleic acid can comprise a

nucleotide sequence encoding at least one, two, three, four, five, or six CDRs or hypervariable loops from heavy and light chain variable regions having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, *e.g.*, conserved substitutions).

In certain embodiments, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a heavy chain variable region having the nucleotide sequence as set forth in the tables herein, a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or capable of hybridizing under the stringency conditions described herein). In another embodiment, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, or three CDRs or hypervariable loops from a light chain variable region having the nucleotide sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or capable of hybridizing under the stringency conditions described herein). In yet another embodiment, the nucleic acid can comprise a nucleotide sequence encoding at least one, two, three, four, five, or six CDRs or hypervariable loops from heavy and light chain variable regions having the nucleotide sequence as set forth in the tables herein, or a sequence substantially homologous thereto (*e.g.*, a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or capable of hybridizing under the stringency conditions described herein).

In another aspect, the application features host cells and vectors containing the nucleic acids described herein. The nucleic acids may be present in a single vector or separate vectors present in the same host cell or separate host cell, as described in more detail herein below.

Vectors

Further provided herein are vectors comprising the nucleotide sequences encoding an antibody molecule described herein. In one embodiment, the vectors comprise nucleotides encoding an antibody molecule described herein. In one embodiment, the vectors comprise the nucleotide sequences described herein. The vectors include, but are not limited to, a virus, plasmid, cosmid, lambda phage or a yeast artificial chromosome (YAC).

Numerous vector systems can be employed. For example, one class of vectors utilizes

DNA elements which are derived from animal viruses such as, for example, bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (Rous Sarcoma Virus, MMTV or MOMLV) or SV40 virus. Another class of vectors utilizes RNA elements derived from RNA viruses such as Semliki Forest virus, Eastern Equine Encephalitis virus and Flaviviruses.

Additionally, cells which have stably integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow for the selection of transfected host cells. The marker may provide, for example, prototrophy to an auxotrophic host, biocide resistance (*e.g.*, antibiotics), or resistance to heavy metals such as copper, or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals.

Once the expression vector or DNA sequence containing the constructs has been prepared for expression, the expression vectors may be transfected or introduced into an appropriate host cell. Various techniques may be employed to achieve this, such as, for example, protoplast fusion, calcium phosphate precipitation, electroporation, retroviral transduction, viral transfection, gene gun, lipid based transfection or other conventional techniques. In the case of protoplast fusion, the cells are grown in media and screened for the appropriate activity.

Methods and conditions for culturing the resulting transfected cells and for recovering the antibody molecule produced are known to those skilled in the art, and may be varied or optimized depending upon the specific expression vector and mammalian host cell employed, based upon the present description.

Cells

In another aspect, the application features host cells and vectors containing the nucleic acids described herein. The nucleic acids may be present in a single vector or separate vectors present in the same host cell or separate host cell. The host cell can be a eukaryotic cell, *e.g.*, a mammalian cell, an insect cell, a yeast cell, or a prokaryotic cell, *e.g.*, *E. coli*. For example, the mammalian cell can be a cultured cell or a cell line. Exemplary mammalian cells include

lymphocytic cell lines (*e.g.*, NSO), Chinese hamster ovary cells (CHO), COS cells, oocyte cells, and cells from a transgenic animal, *e.g.*, mammary epithelial cell.

The invention also provides host cells comprising a nucleic acid encoding an antibody molecule as described herein.

In one embodiment, the host cells are genetically engineered to comprise nucleic acids encoding the antibody molecule.

In one embodiment, the host cells are genetically engineered by using an expression cassette. The phrase “expression cassette,” refers to nucleotide sequences, which are capable of affecting expression of a gene in hosts compatible with such sequences. Such cassettes may include a promoter, an open reading frame with or without introns, and a termination signal. Additional factors necessary or helpful in effecting expression may also be used, such as, for example, an inducible promoter.

The invention also provides host cells comprising the vectors described herein.

The cell can be, but is not limited to, a eukaryotic cell, a bacterial cell, an insect cell, or a human cell. Suitable eukaryotic cells include, but are not limited to, Vero cells, HeLa cells, COS cells, CHO cells, HEK293 cells, BHK cells and MDCKII cells. Suitable insect cells include, but are not limited to, Sf9 cells.

Uses and Combination Therapies

The multispecific molecule described herein, alone or in combination with a second therapy or a second therapeutic agent, can be used to treat a hyperproliferative disorder, a cancer, or a fibrotic disorder.

Cancer

Methods described herein include treating a cancer in a subject by using a multispecific molecule described herein, *e.g.*, using a pharmaceutical composition described herein. Also provided are methods for reducing or ameliorating a symptom of a cancer in a subject, as well as methods for inhibiting the growth of a cancer and/or killing one or more cancer cells. In embodiments, the methods described herein decrease the size of a tumor and/or decrease the number of cancer cells in a subject administered with a described herein or a pharmaceutical composition described herein.

In embodiments, the cancer is a hematological cancer. In embodiments, the hematological cancer is a leukemia or a lymphoma. As used herein, a “hematologic cancer” refers to a tumor of the hematopoietic or lymphoid tissues, *e.g.*, a tumor that affects blood, bone marrow, or lymph nodes. Exemplary hematologic malignancies include, but are not limited to, leukemia (*e.g.*, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia, acute monocytic leukemia (AMoL), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), or large granular lymphocytic leukemia), lymphoma (*e.g.*, AIDS-related lymphoma, cutaneous T-cell lymphoma, Hodgkin lymphoma (*e.g.*, classical Hodgkin lymphoma or nodular lymphocyte-predominant Hodgkin lymphoma), mycosis fungoides, non-Hodgkin lymphoma (*e.g.*, B-cell non-Hodgkin lymphoma (*e.g.*, Burkitt lymphoma, small lymphocytic lymphoma (CLL/SLL), diffuse large B-cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, or mantle cell lymphoma) or T-cell non-Hodgkin lymphoma (mycosis fungoides, anaplastic large cell lymphoma, or precursor T-lymphoblastic lymphoma)), primary central nervous system lymphoma, Sézary syndrome, Waldenström macroglobulinemia), chronic myeloproliferative neoplasm, Langerhans cell histiocytosis, multiple myeloma/plasma cell neoplasm, myelodysplastic syndrome, or myelodysplastic/myeloproliferative neoplasm.

In embodiments, the cancer is a solid cancer. Exemplary solid cancers include, but are not limited to, ovarian cancer, rectal cancer, stomach cancer, testicular cancer, cancer of the anal region, uterine cancer, colon cancer, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, Kaposi's sarcoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, brain stem glioma, pituitary adenoma, epidermoid cancer, carcinoma of the cervix squamous cell cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the vagina, sarcoma of soft tissue, cancer of the urethra, carcinoma of the vulva, cancer of the penis, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, spinal axis tumor, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, metastatic lesions of said cancers, or combinations thereof.

In certain embodiments, the cancer is an epithelial, mesenchymal or hematologic malignancy. In certain embodiments, the cancer treated is a solid tumor (e.g., carcinoid, carcinoma or sarcoma), a soft tissue tumor (e.g., a heme malignancy), and a metastatic lesion, e.g., a metastatic lesion of any of the cancers disclosed herein. In one embodiment, the cancer treated is a fibrotic or desmoplastic solid tumor, e.g., a tumor having one or more of: limited tumor perfusion, compressed blood vessels, fibrotic tumor interstitium, or increased interstitial fluid pressure. In one embodiment, the solid tumor is chosen from one or more of pancreatic (e.g., pancreatic adenocarcinoma or pancreatic ductal adenocarcinoma), breast, colon, colorectal, lung (e.g., small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC)), skin, ovarian, liver cancer, esophageal cancer, endometrial cancer, gastric cancer, head and neck cancer, kidney, or prostate cancer.

Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers are noted below and include: squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial cancer or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer. The term “cancer” includes primary malignant cells or tumors (e.g., those whose cells have not migrated to sites in the subject's body other than the site of the original malignancy or tumor) and secondary malignant cells or tumors (e.g., those arising from metastasis, the migration of malignant cells or tumor cells to secondary sites that are different from the site of the original tumor).

Other examples of cancers or malignancies include, but are not limited to: Acute Childhood Lymphoblastic Leukemia, Acute Lymphoblastic Leukemia, Acute Lymphocytic Leukemia, Acute Myeloid Leukemia, Adrenocortical Carcinoma, Adult (Primary) Hepatocellular Cancer, Adult (Primary) Liver Cancer, Adult Acute Lymphocytic Leukemia, Adult Acute Myeloid Leukemia, Adult Hodgkin's Disease, Adult Hodgkin's Lymphoma, Adult Lymphocytic Leukemia, Adult Non-Hodgkin's Lymphoma, Adult Primary Liver Cancer, Adult Soft Tissue

Sarcoma, AIDS-Related Lymphoma, AIDS-Related Malignancies, Anal Cancer, Astrocytoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain Stem Glioma, Brain Tumors, Breast Cancer, Cancer of the Renal Pelvis and Ureter, Central Nervous System (Primary) Lymphoma, Central Nervous System Lymphoma, Cerebellar Astrocytoma, Cerebral Astrocytoma, Cervical Cancer, Childhood (Primary) Hepatocellular Cancer, Childhood (Primary) Liver Cancer, Childhood Acute Lymphoblastic Leukemia, Childhood Acute Myeloid Leukemia, Childhood Brain Stem Glioma, Childhood Cerebellar Astrocytoma, Childhood Cerebral Astrocytoma, Childhood Extracranial Germ Cell Tumors, Childhood Hodgkin's Disease, Childhood Hodgkin's Lymphoma, Childhood Hypothalamic and Visual Pathway Glioma, Childhood Lymphoblastic Leukemia, Childhood Medulloblastoma, Childhood Non-Hodgkin's Lymphoma, Childhood Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood Primary Liver Cancer, Childhood Rhabdomyosarcoma, Childhood Soft Tissue Sarcoma, Childhood Visual Pathway and Hypothalamic Glioma, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Colon Cancer, Cutaneous T-Cell Lymphoma, Endocrine Pancreas Islet Cell Carcinoma, Endometrial Cancer, Ependymoma, Epithelial Cancer, Esophageal Cancer, Ewing's Sarcoma and Related Tumors, Exocrine Pancreatic Cancer, Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer, Female Breast Cancer, Gaucher's Disease, Gallbladder Cancer, Gastric Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Tumors, Germ Cell Tumors, Gestational Trophoblastic Tumor, Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular Cancer, Hodgkin's Disease, Hodgkin's Lymphoma, Hypergammaglobulinemia, Hypopharyngeal Cancer, Intestinal Cancers, Intraocular Melanoma, Islet Cell Carcinoma, Islet Cell Pancreatic Cancer, Kaposi's Sarcoma, Kidney Cancer, Laryngeal Cancer, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer, Lymphoproliferative Disorders, Macroglobulinemia, Male Breast Cancer, Malignant Mesothelioma, Malignant Thymoma, Medulloblastoma, Melanoma, Mesothelioma, Metastatic Occult Primary Squamous Neck Cancer, Metastatic Primary Squamous Neck Cancer, Metastatic Squamous Neck Cancer, Multiple Myeloma, Multiple Myeloma/Plasma Cell Neoplasm, Myelodysplastic Syndrome, Myelogenous Leukemia, Myeloid Leukemia, Myeloproliferative Disorders, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin's Lymphoma During Pregnancy, Nonmelanoma Skin Cancer, Non-Small Cell Lung Cancer, Occult Primary Metastatic Squamous Neck Cancer, Oropharyngeal Cancer, Osteo-/Malignant Fibrous Sarcoma,

Osteosarcoma/Malignant Fibrous Histiocytoma, Osteosarcoma/Malignant Fibrous Histiocytoma of Bone, Ovarian Epithelial Cancer, Ovarian Germ Cell Tumor, Ovarian Low Malignant Potential Tumor, Pancreatic Cancer, Paraproteinemias, Purpura, Parathyroid Cancer, Penile Cancer, Pheochromocytoma, Pituitary Tumor, Plasma Cell Neoplasm/Multiple Myeloma, Primary Central Nervous System Lymphoma, Primary Liver Cancer, Prostate Cancer, Rectal Cancer, Renal Cell Cancer, Renal Pelvis and Ureter Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoidosis Sarcomas, Sezary Syndrome, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Neck Cancer, Stomach Cancer, Supratentorial Primitive Neuroectodermal and Pineal Tumors, T-Cell Lymphoma, Testicular Cancer, Thymoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Transitional Renal Pelvis and Ureter Cancer, Trophoblastic Tumors, Ureter and Renal Pelvis Cell Cancer, Urethral Cancer, Uterine Cancer, Uterine Sarcoma, Vaginal Cancer, Visual Pathway and Hypothalamic Glioma, Vulvar Cancer, Waldenstrom's Macroglobulinemia, Wilms' Tumor, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

In other embodiments, the multispecific molecule, as described above and herein, is used to treat a hyperproliferative disorder, e.g., a hyperproliferative connective tissue disorder (e.g., a hyperproliferative fibrotic disease). In one embodiment, the hyperproliferative fibrotic disease is multisystemic or organ-specific. Exemplary hyperproliferative fibrotic diseases include, but are not limited to, multisystemic (e.g., systemic sclerosis, multifocal fibrosclerosis, sclerodermatous graft-versus-host disease in bone marrow transplant recipients, nephrogenic systemic fibrosis, scleroderma), and organ-specific disorders (e.g., fibrosis of the eye, lung, liver, heart, kidney, pancreas, skin and other organs). In other embodiments, the disorder is chosen from liver cirrhosis or tuberculosis. In other embodiments, the disorder is leprosy.

In embodiments, the multispecific molecules (or pharmaceutical composition) are administered in a manner appropriate to the disease to be treated or prevented. The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease. Appropriate dosages may be determined by clinical trials. For example, when "an effective amount" or "a therapeutic amount" is indicated, the precise amount of the pharmaceutical composition (or multispecific molecules) to be administered can be determined by a physician with consideration of individual differences in

tumor size, extent of infection or metastasis, age, weight, and condition of the subject. In embodiments, the pharmaceutical composition described herein can be administered at a dosage of 10^4 to 10^9 cells/kg body weight, e.g., 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. In embodiments, the pharmaceutical composition described herein can be administered multiple times at these dosages. In embodiments, the pharmaceutical composition described herein can be administered using infusion techniques described in immunotherapy (see, e.g., Rosenberg et al., New Eng. J. of Med. 319:1676, 1988).

In embodiments, the multispecific molecules or pharmaceutical composition is administered to the subject parenterally. In embodiments, the cells are administered to the subject intravenously, subcutaneously, intratumorally, intranodally, intramuscularly, intradermally, or intraperitoneally. In embodiments, the cells are administered, e.g., injected, directly into a tumor or lymph node. In embodiments, the cells are administered as an infusion (e.g., as described in Rosenberg et al., New Eng. J. of Med. 319:1676, 1988) or an intravenous push. In embodiments, the cells are administered as an injectable depot formulation. In embodiments, the subject is a mammal. In embodiments, the subject is a human, monkey, pig, dog, cat, cow, sheep, goat, rabbit, rat, or mouse. In embodiments, the subject is a human. In embodiments, the subject is a pediatric subject, e.g., less than 18 years of age, e.g., less than 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or less years of age. In embodiments, the subject is an adult, e.g., at least 18 years of age, e.g., at least 19, 20, 21, 22, 23, 24, 25, 25-30, 30-35, 35-40, 40-50, 50-60, 60-70, 70-80, or 80-90 years of age.

Liver Conditions or Disorders

This invention also provides methods of treating liver conditions or disorders using the multispecific molecules or pharmaceutical compositions described herein.

As used herein, “liver disorder therapy” refers to therapies or therapeutic agents used to treat or prevent a liver disorder described herein, and therefore encompasses liver cancer therapies and other liver disorder therapies, e.g., therapies for fibrotic liver disorders, fatty liver diseases, liver inflammation disorders, autoimmune liver diseases, and liver disorders induced by genetic diseases, alcoholism, drug toxicity, infection, or injury.

Examples of liver cancers include: hepatocellular carcinoma (HCC), primary liver cell carcinoma, hepatoma, fibrolamellar carcinoma, focal nodular hyperplasia, cholangiosarcoma,

intrahepatic bile duct cancer, angiosarcoma or hemangiosarcoma, hepatic adenoma, hepatic hemangiomas, hepatic hamartoma, hepatoblastoma, infantile hemangioendothelialoma, mixed tumors of the liver, tumors of mesenchymal tissue, sarcoma of the liver. Examples of cancers that may metastasize to the liver include: breast cancer, colorectal cancer, esophageal cancer, kidney or renal cancer, lung cancer, ovarian cancer, pancreatic cancer, rectal cancer, skin cancer (e.g., melanoma), gastric or stomach cancer (including gastrointestinal cancer), and uterine cancer.

In an embodiment, the liver disorder is a fibrotic disorder or connective tissue disorder affecting the function or physiology of the liver. In one embodiment, the fibrotic disorder or connective tissue disorder can be systemic (affecting the whole body), multi-organ, or organ-specific (e.g., liver-specific). Examples of fibrotic liver disorders include liver fibrosis (hepatic fibrosis), liver cirrhosis, and any disorder associated with accumulation of extracellular matrix proteins, e.g., collagen, in the liver, liver scarring, and/or abnormal hepatic vasculature. Liver fibrosis is caused by liver inflammation or damage which triggers the accumulation of extracellular matrix proteins, including collagens, and scar tissue in the liver. Liver cirrhosis is the end stage of liver fibrosis, involves regenerative nodules (as a result of repair processes), and is accompanied with the distortion of the hepatic vasculature. Liver fibrotic disorders are most commonly caused by chronic viral infection (e.g., hepatitis B, hepatitis C), alcoholism, and fatty liver disease.

Examples of fatty liver diseases include fatty liver (or FLD), alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcoholic steatohepatitis, simple steatosis, Reye's syndrome, and any disorder associated with abnormal retention of lipids in liver cells.

In one embodiment, the liver disease is NASH.

Metabolic disorders can also affect the liver and cause liver damage. Examples of metabolic disorders of the liver or affecting the liver include hemochromatosis, diabetes, obesity, hypertension, dyslipidemia, galactosemia, and glycogen storage disease.

Autoimmune disorders of the liver or affecting the liver can include systemic disorders or disorders that primarily affect an organ other than the liver, but with secondary effects to liver cells or liver function. Examples of such autoimmune disorders include autoimmune hepatitis

(AIH), autoimmune liver disease, lupoid hepatitis, systemic lupus erythematosus, primary biliary cirrhosis (PBC), scleroderma, and systemic sclerosis.

Fibrotic Conditions or Disorders

In another aspect, the invention features a method of treating or preventing a fibrotic condition or disorder in a subject. The method includes administering the multispecific molecule, as a single agent or in combination with another agent or therapeutic modality, to a subject in need thereof, in an amount sufficient to decrease or inhibit the fibrotic condition in the subject.

In certain embodiments, reducing fibrosis, or treatment of a fibrotic condition, includes reducing or inhibiting one or more of: formation or deposition of tissue fibrosis; reducing the size, cellularity (e.g., fibroblast or immune cell numbers), composition; or cellular content, of a fibrotic lesion; reducing the collagen or hydroxyproline content, of a fibrotic lesion; reducing expression or activity of a fibrogenic protein; reducing fibrosis associated with an inflammatory response; decreasing weight loss associated with fibrosis; or increasing survival.

In certain embodiments, the fibrotic condition is primary fibrosis. In one embodiment, the fibrotic condition is idiopathic. In other embodiments, the fibrotic condition is associated with (e.g., is secondary to) a disease (e.g., an infectious disease, an inflammatory disease, an autoimmune disease, a malignant or cancerous disease, and/or a connective disease); a toxin; an insult (e.g., an environmental hazard (e.g., asbestos, coal dust, polycyclic aromatic hydrocarbons), cigarette smoking, a wound); a medical treatment (e.g., surgical incision, chemotherapy or radiation), or a combination thereof.

In certain embodiments, the fibrotic condition is a fibrotic condition of the lung, a fibrotic condition of the liver (e.g., as described herein), a fibrotic condition of the heart or vasculature, a fibrotic condition of the kidney, a fibrotic condition of the skin, a fibrotic condition of the gastrointestinal tract, a fibrotic condition of the bone marrow or a hematopoietic tissue, a fibrotic condition of the nervous system, a fibrotic condition of the eye, or a combination thereof.

In certain embodiments, the fibrotic condition is a fibrotic condition of the lung. In certain embodiments, the fibrotic condition of the lung is chosen from one or more of: pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), usual interstitial pneumonitis (UIP), interstitial lung disease, cryptogenic fibrosing alveolitis (CFA), bronchiectasis, and scleroderma

lung disease. In one embodiment, the fibrosis of the lung is secondary to a disease, a toxin, an insult, a medical treatment, or a combination thereof. For example, the fibrosis of the lung can be associated with (e.g., secondary to) one or more of: a disease process such as asbestosis and silicosis; an occupational hazard; an environmental pollutant; cigarette smoking; an autoimmune connective tissue disorders (e.g., rheumatoid arthritis, scleroderma and systemic lupus erythematosus (SLE)); a connective tissue disorder such as sarcoidosis; an infectious disease, e.g., infection, particularly chronic infection; a medical treatment, including but not limited to, radiation therapy, and drug therapy, e.g., chemotherapy (e.g., treatment with as bleomycin, methotrexate, amiodarone, busulfan, and/or nitrofurantoin). In one embodiment, the fibrotic condition of the lung treated with the methods of the invention is associated with (e.g., secondary to) a cancer treatment, e.g., treatment of a cancer (e.g., squamous cell carcinoma, testicular cancer, Hodgkin's disease with bleomycin). In one embodiment, the fibrotic condition of the lung is associated with an autoimmune connective tissue disorder (e.g., scleroderma or lupus, e.g., SLE).

Pulmonary fibrosis can occur as a secondary effect in disease processes such as asbestosis and silicosis, and is known to be more prevalent in certain occupations such as coal miner, ship workers and sand blasters where exposure to environmental pollutants is an occupational hazard (Green, FH et al. (2007) *Toxicol Pathol.* 35:136-47). Other factors that contribute to pulmonary fibrosis include cigarette smoking, and autoimmune connective tissue disorders, like rheumatoid arthritis, scleroderma and systemic lupus erythematosus (SLE) (Leslie, KO et al. (2007) *Semin Respir Crit Care Med.* 28:369-78; Swigris, JJ et al. (2008) *Chest.* 133:271-80; and Antoniou, KM et al. (2008) *Curr Opin Rheumatol.* 20:686-91). Other connective tissue disorders such as sarcoidosis can include pulmonary fibrosis as part of the disease (Paramothayan, S et al. (2008) *Respir Med.* 102:1-9), and infectious diseases of the lung can cause fibrosis as a long term consequence of infection, particularly chronic infections.

Pulmonary fibrosis can also be a side effect of certain medical treatments, particularly radiation therapy to the chest and certain medicines like bleomycin, methotrexate, amiodarone, busulfan, and nitrofurantoin (Catane, R et al. (1979) *Int J Radiat Oncol Biol Phys.* 5:1513-8; Zisman, DA et al. (2001) *Sarcoidosis Vasc Diffuse Lung Dis.* 18:243-52; Rakita, L et al. (1983) *Am Heart J.* 106:906-16; Twohig, KJ et al. (1990) *Clin Chest Med.* 11:31-54; and Witten CM. (1989) *Arch Phys Med Rehabil.* 70:55-7). In other embodiments, idiopathic pulmonary fibrosis

can occur where no clear causal agent or disease can be identified. Genetic factors can play a significant role in these cases of pulmonary fibrosis (Steele, MP et al. (2007) *Respiration* 74:601-8; Brass, DM et al. (2007) *Proc Am Thorac Soc.* 4:92-100 and du Bois RM. (2006) *Semin Respir Crit Care Med.* 27:581-8).

In other embodiments, pulmonary fibrosis includes, but is not limited to, pulmonary fibrosis associated with chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome, scleroderma, pleural fibrosis, chronic asthma, acute lung syndrome, amyloidosis, bronchopulmonary dysplasia, Caplan's disease, Dressler's syndrome, histiocytosis X, idiopathic pulmonary haemosiderosis, lymphangiomyomatosis, mitral valve stenosis, polymyositis, pulmonary edema, pulmonary hypertension (e.g., idiopathic pulmonary hypertension (IPH)), pneumoconiosis, radiotherapy (e.g., radiation induced fibrosis), rheumatoid disease, Shaver's disease, systemic lupus erythematosus, systemic sclerosis, tropical pulmonary eosinophilia, tuberous sclerosis, Weber-Christian disease, Wegener's granulomatosis, Whipple's disease, or exposure to toxins or irritants (e.g., pharmaceutical drugs such as amiodarone, bleomycin, busulphan, carmustine, chloramphenicol, hexamethonium, methotrexate, methysergide, mitomycin C, nitrofurantoin, penicillamine, peplomycin, and practolol; inhalation of talc or dust, e.g., coal dust, silica). In certain embodiments, the pulmonary fibrosis is associated with an inflammatory disorder of the lung, e.g., asthma, and/or COPD.

In certain embodiments, the fibrotic condition is a fibrotic condition of the liver. In certain embodiments, the fibrotic condition of the liver is chosen from one or more of: fatty liver disease, steatosis (e.g., nonalcoholic steatohepatitis (NASH), cholestatic liver disease (e.g., primary biliary cirrhosis (PBC)), cirrhosis, alcohol induced liver fibrosis, biliary duct injury, biliary fibrosis, or cholangiopathies. In other embodiments, hepatic or liver fibrosis includes, but is not limited to, hepatic fibrosis associated with alcoholism, viral infection, e.g., hepatitis (e.g., hepatitis C, B or D), autoimmune hepatitis, non-alcoholic fatty liver disease (NAFLD), progressive massive fibrosis, exposure to toxins or irritants (e.g., alcohol, pharmaceutical drugs and environmental toxins). Additional examples of liver conditions and disorders are provided in the Sections entitled "Liver Conditions or Disorders," provided herein.

In certain embodiments, the fibrotic condition is a fibrotic condition of the kidney. In certain embodiments, the fibrotic condition of the kidney is chosen from one or more of: renal fibrosis (e.g., chronic kidney fibrosis), nephropathies associated with injury/fibrosis (e.g.,

chronic nephropathies associated with diabetes (e.g., diabetic nephropathy)), lupus, scleroderma of the kidney, glomerular nephritis, focal segmental glomerular sclerosis, IgA nephropathy renal fibrosis associated with human chronic kidney disease (CKD), chronic progressive nephropathy (CPN), tubulointerstitial fibrosis, ureteral obstruction, chronic uremia, chronic interstitial nephritis, radiation nephropathy, glomerulosclerosis, progressive glomerulonephrosis (PGN), endothelial/thrombotic microangiopathy injury, HIV-associated nephropathy, or fibrosis associated with exposure to a toxin, an irritant, or a chemotherapeutic agent. In one embodiment, the fibrotic condition of the kidney is scleroderma of the kidney. In some embodiments, the fibrotic condition of the kidney is transplant nephropathy, diabetic nephropathy, lupus nephritis, focal segmental glomerulosclerosis (FSGS), endothelial/thrombotic microangiopathy injury, scleroderma of the kidney, HIV-associated nephropathy (HIVVAN), or exposure to toxins, irritants, chemotherapeutic agents.

In certain embodiments, the fibrotic condition is a fibrotic condition of the bone marrow or a hematopoietic tissue. In certain embodiments, the fibrotic condition of the bone marrow is an intrinsic feature of a chronic myeloproliferative neoplasm of the bone marrow, such as primary myelofibrosis (also referred to herein as agnogenic myeloid metaplasia or chronic idiopathic myelofibrosis). In other embodiments, the bone marrow fibrosis is associated with (e.g., is secondary to) a malignant condition or a condition caused by a clonal proliferative disease. In other embodiments, the bone marrow fibrosis is associated with a hematologic disorder (e.g., a hematologic disorder chosen from one or more of polycythemia vera, essential thrombocythemia, myelodysplasia, hairy cell leukemia, lymphoma (e.g., Hodgkin or non-Hodgkin lymphoma), multiple myeloma or chronic myelogenous leukemia (CML)). In yet other embodiments, the bone marrow fibrosis is associated with (e.g., secondary to) a non-hematologic disorder (e.g., a non-hematologic disorder chosen from solid tumor metastasis to bone marrow, an autoimmune disorder (e.g., systemic lupus erythematosus, scleroderma, mixed connective tissue disorder, or polymyositis), an infection (e.g., tuberculosis or leprosy), or secondary hyperparathyroidism associated with vitamin D deficiency. In some embodiments, the fibrotic condition is idiopathic or drug-induced myelofibrosis. In some embodiments, the fibrotic condition of the bone marrow or hematopoietic tissue is associated with systemic lupus erythematosus or scleroderma.

In other embodiments, the fibrotic condition is associated with leprosy or tuberculosis.

In certain embodiments, the fibrotic condition is a fibrotic condition of the bone marrow. In certain embodiments, the fibrotic condition of the bone marrow is myelofibrosis (e.g., primary myelofibrosis (PMF)), myeloid metaplasia, chronic idiopathic myelofibrosis, or primary myelofibrosis. In other embodiments, bone marrow fibrosis is associated with a hematologic disorder chosen from one or more of hairy cell leukemia, lymphoma, or multiple myeloma.

In other embodiments, the bone marrow fibrosis is associated with one or more myeloproliferative neoplasms (MPN) chosen from: essential thrombocythemia (ET), polycythemia vera (PV), mastocytosis, chronic eosinophilic leukemia, chronic neutrophilic leukemia, or other MPN.

In one embodiment, the fibrotic condition is primary myelofibrosis. Primary myelofibrosis (PMF) (also referred to in the literature as idiopathic myeloid metaplasia, and Agnogenic myeloid metaplasia) is a clonal disorder of multipotent hematopoietic progenitor cells (reviewed in Abdel-Wahab, O. et al. (2009) *Annu. Rev. Med.* 60:233-45; Varicchio, L. et al. (2009) *Expert Rev. Hematol.* 2(3):315-334; Agrawal, M. et al. (2010) *Cancer* 1-15).

In certain embodiments, the fibrotic condition is a fibrotic condition of the heart. In certain embodiments, the fibrotic condition of the heart is myocardial fibrosis (e.g., myocardial fibrosis associated with radiation myocarditis, a surgical procedure complication (e.g., myocardial post-operative fibrosis), infectious diseases (e.g., Chagas disease, bacterial, trichinosis or fungal myocarditis)); granulomatous, metabolic storage disorders (e.g., cardiomyopathy, hemochromatosis); developmental disorders (e.g., endocardial fibroelastosis); arteriosclerotic, or exposure to toxins or irritants (e.g., drug induced cardiomyopathy, drug induced cardiotoxicity, alcoholic cardiomyopathy, cobalt poisoning or exposure). In certain embodiments, the myocardial fibrosis is associated with an inflammatory disorder of cardiac tissue (e.g., myocardial sarcoidosis). In some embodiments, the fibrotic condition is a fibrotic condition associated with a myocardial infarction. In some embodiments, the fibrotic condition is a fibrotic condition associated with congestive heart failure.

In some embodiments, the fibrotic condition is associated with an autoimmune disease selected from scleroderma or lupus, e.g., systemic lupus erythematosus.

In some embodiments, the fibrotic condition is systemic. In some embodiments, the fibrotic condition is systemic sclerosis (e.g., limited systemic sclerosis, diffuse systemic

sclerosis, or systemic sclerosis sine scleroderma), nephrogenic systemic fibrosis, cystic fibrosis, chronic graft vs. host disease, or atherosclerosis.

In some embodiments, the fibrotic condition is scleroderma. In some embodiments, the scleroderma is localized, e.g., morphea or linear scleroderma. In some embodiments, the condition is a systemic sclerosis, e.g., limited systemic sclerosis, diffuse systemic sclerosis, or systemic sclerosis sine scleroderma.

In other embodiment, the fibrotic condition affects a tissue chosen from one or more of muscle, tendon, cartilage, skin (e.g., skin epidermis or endodermis), cardiac tissue, vascular tissue (e.g., artery, vein), pancreatic tissue, lung tissue, liver tissue, kidney tissue, uterine tissue, ovarian tissue, neural tissue, testicular tissue, peritoneal tissue, colon, small intestine, biliary tract, gut, bone marrow, hematopoietic tissue, or eye (e.g., retinal) tissue.

In some embodiments, the fibrotic condition is a fibrotic condition of the eye. In some embodiments, the fibrotic condition is glaucoma, macular degeneration (e.g., age-related macular degeneration), macular edema (e.g., diabetic macular edema), retinopathy (e.g., diabetic retinopathy), or dry eye disease.

In certain embodiments, the fibrotic condition is a fibrotic condition of the skin. In certain embodiments, the fibrotic condition of the skin is chosen from one or more of: skin fibrosis (e.g., hypertrophic scarring, keloid), scleroderma, nephrogenic systemic fibrosis (e.g., resulting after exposure to gadolinium (which is frequently used as a contrast substance for MRIs) in patients with severe kidney failure), and keloid.

In certain embodiments, the fibrotic condition is a fibrotic condition of the gastrointestinal tract. In certain embodiments, the fibrotic condition is chosen from one or more of: fibrosis associated with scleroderma; radiation induced gut fibrosis; fibrosis associated with a foregut inflammatory disorder such as Barrett's esophagus and chronic gastritis, and/or fibrosis associated with a hindgut inflammatory disorder, such as inflammatory bowel disease (IBD), ulcerative colitis and Crohn's disease. In some embodiments, the fibrotic condition of the gastrointestinal tract is fibrosis associated with scleroderma.

In one embodiment, the fibrotic condition is a chronic fibrotic condition or disorder. In certain embodiments, the fibrotic condition is associated with an inflammatory condition or disorder.

In some embodiments, the fibrotic and/or inflammatory condition is osteomyelitis, e.g., chronic osteomyelitis.

In some embodiments, the fibrotic condition is an amyloidosis. In certain embodiments, the amyloidosis is associated with chronic osteomyelitis.

In some embodiments, the one or more compositions described herein is administered in combination with one or more other therapeutic agents. Exemplary therapeutic agents include, but are not limited to, anti-fibrotics, corticosteroids, antiinflammatories, immunosuppressants, chemotherapeutic agents, anti-metabolites, and immunomodulators.

An example of suitable therapeutics for use in combination with the composition(s) for treatment of liver fibrosis includes, but is not limited to, adefovir dipivoxil, candesartan, colchicine, combined ATG, mycophenolate mofetil, and tacrolimus, combined cyclosporine microemulsion and tacrolimus, elastometry, everolimus, FG-3019, Fuzheng Huayu, GI262570, glycyrrhizin (monoammonium glycyrrhizinate, glycine, L-cysteine monohydrochloride), interferon gamma-1b, irbesartan, losartan, oltipraz, ORAL IMPACT®, peginterferon alfa-2a, combined peginterferon alfa-2a and ribavirin, peginterferon alfa-2b (SCH 54031), combined peginterferon alpha-2b and ribavirin, praziquantel, prazosin, raltegravir, ribavirin (REBETOL®, SCH 18908), ritonavir-boosted protease inhibitor, pentoxifylline, tacrolimus, tauroursodeoxycholic acid, tocopherol, ursodiol, warfarin, and combinations thereof.

Combination Therapies

The multispecific molecules disclosed herein can be used in combination with a second therapeutic agent or procedure.

In embodiments, the multispecific molecule and the second therapeutic agent or procedure are administered/performed after a subject has been diagnosed with a cancer, e.g., before the cancer has been eliminated from the subject. In embodiments, the multispecific molecule and the second therapeutic agent or procedure are administered/performed simultaneously or concurrently. For example, the delivery of one treatment is still occurring when the delivery of the second commences, e.g., there is an overlap in administration of the treatments. In other embodiments, the multispecific molecule and the second therapeutic agent or procedure are administered/performed sequentially. For example, the delivery of one treatment ceases before the delivery of the other treatment begins.

In embodiments, combination therapy can lead to more effective treatment than monotherapy with either agent alone. In embodiments, the combination of the first and second treatment is more effective (e.g., leads to a greater reduction in symptoms and/or cancer cells) than the first or second treatment alone. In embodiments, the combination therapy permits use of a lower dose of the first or the second treatment compared to the dose of the first or second treatment normally required to achieve similar effects when administered as a monotherapy. In embodiments, the combination therapy has a partially additive effect, wholly additive effect, or greater than additive effect.

In one embodiment, the multispecific molecule is administered in combination with a therapy, *e.g.*, a cancer therapy (*e.g.*, one or more of anti-cancer agents, immunotherapy, photodynamic therapy (PDT), surgery and/or radiation). The terms “chemotherapeutic,” “chemotherapeutic agent,” and “anti-cancer agent” are used interchangeably herein. The administration of the multispecific molecule and the therapy, *e.g.*, the cancer therapy, can be sequential (with or without overlap) or simultaneous. Administration of the multispecific molecule can be continuous or intermittent during the course of therapy (*e.g.*, cancer therapy). Certain therapies described herein can be used to treat cancers and non-cancerous diseases. For example, PDT efficacy can be enhanced in cancerous and non-cancerous conditions (*e.g.*, tuberculosis) using the methods and compositions described herein (reviewed in, *e.g.*, Agostinis, P. *et al.* (2011) *CA Cancer J. Clin.* 61:250-281).

Anti-cancer therapies

In other embodiments, the multispecific molecule is administered in combination with a low or small molecular weight chemotherapeutic agent. Exemplary low or small molecular weight chemotherapeutic agents include, but not limited to, 13-cis-retinoic acid (isotretinoin, ACCUTANE®), 2-CdA (2-chlorodeoxyadenosine, cladribine, LEUSTATIN™), 5-azacitidine (azacitidine, VIDAZA®), 5-fluorouracil (5-FU, fluorouracil, ADRUCIL®), 6-mercaptopurine (6-MP, mercaptopurine, PURINETHOL®), 6-TG (6-thioguanine, thioguanine, THIOGUANINE TABLOID®), abraxane (paclitaxel protein-bound), actinomycin-D (dactinomycin, COSMEGEN®), alitretinoin (PANRETIN®), all-transretinoic acid (ATRA, tretinoin, VESANOID®), altretamine (hexamethylmelamine, HMM, HEXALEN®), amethopterin (methotrexate, methotrexate sodium, MTX, TREXALL™, RHEUMATREX®), amifostine

(ETHYOL®), arabinosylcytosine (Ara-C, cytarabine, CYTOSAR-U®), arsenic trioxide (TRISENOX®), asparaginase (Erwinia L-asparaginase, L-asparaginase, ELSPAR®, KIDROLASE®), BCNU (carmustine, BiCNU®), bendamustine (TREANDA®), bexarotene (TARGRETIN®), bleomycin (BLENOXANE®), busulfan (BUSULFEX®, MYLERAN®), calcium leucovorin (Citrovorum Factor, folinic acid, leucovorin), camptothecin-11 (CPT-11, irinotecan, CAMPTOSAR®), capecitabine (XELODA®), carboplatin (PARAPLATIN®), carmustine wafer (proliferospa 20 with carmustine implant, GLIADEL® wafer), CCI-779 (temsirolimus, TORISEL®), CCNU (lomustine, CeeNU), CDDP (cisplatin, PLATINOL®, PLATINOL-AQ®), chlorambucil (leukeran), cyclophosphamide (CYTOXAN®, NEOSAR®), dacarbazine (DIC, DTIC, imidazole carboxamide, DTIC-DOME®), daunomycin (daunorubicin, daunorubicin hydrochloride, rubidomycin hydrochloride, CERUBIDINE®), decitabine (DACOGEN®), dexrazoxane (ZINECARD®), DHAD (mitoxantrone, NOVANTRONE®), docetaxel (TAXOTERE®), doxorubicin (ADRIAMYCIN®, RUBEX®), epirubicin (ELLENCE™), estramustine (EMCYT®), etoposide (VP-16, etoposide phosphate, TOPOSAR®, VEPESID®, ETOPOPHOS®), floxuridine (FUDR®), fludarabine (FLUDARA®), fluorouracil (cream) (CARACT™, EFUDEX®, FLUOROPLEX®), gemcitabine (GEMZAR®), hydroxyurea (HYDREA®, DROXIA™, MYLOCEL™), idarubicin (IDAMYCIN®), ifosfamide (IFEX®), ixabepilone (IXEMPRA™), LCR (leurocristine, vincristine, VCR, ONCOVIN®, VINCASAR PFS®), L-PAM (L-sarcolysin, melphalan, phenylalanine mustard, ALKERAN®), mechlorethamine (mechlorethamine hydrochloride, mustine, nitrogen mustard, MUSTARGEN®), mesna (MESNEX™), mitomycin (mitomycin-C, MTC, MUTAMYCIN®), nelarabine (ARRANON®), oxaliplatin (ELOXATIN™), paclitaxel (TAXOL®, ONXAL™), pegaspargase (PEG-L-asparaginase, ONCOSPAR®), PEMETREXED (ALIMTA®), pentostatin (NIPENT®), procarbazine (MATULANE®), streptozocin (ZANOSAR®), temozolomide (TEMODAR®), teniposide (VM-26, VUMON®), TESPA (thiophosphoamide, thiotepa, TSPA, THIOPLEX®), topotecan (HYCAMTIN®), vinblastine (vinblastine sulfate, vincaleukoblastine, VLB, ALKABAN-AQ®, VELBAN®), vinorelbine (vinorelbine tartrate, NAVELBINE®), and vorinostat (ZOLINZA®).

In another embodiment, the multispecific molecule is administered in conjunction with a biologic. Biologics useful in the treatment of cancers are known in the art and a binding molecule of the invention may be administered, for example, in conjunction with such known

biologics. For example, the FDA has approved the following biologics for the treatment of breast cancer: HERCEPTIN® (trastuzumab, Genentech Inc., South San Francisco, Calif.; a humanized monoclonal antibody that has anti-tumor activity in HER2-positive breast cancer); FASLODEX® (fulvestrant, AstraZeneca Pharmaceuticals, LP, Wilmington, Del.; an estrogen-receptor antagonist used to treat breast cancer); ARIMIDEX® (anastrozole, AstraZeneca Pharmaceuticals, LP; a nonsteroidal aromatase inhibitor which blocks aromatase, an enzyme needed to make estrogen); Aromasin® (exemestane, Pfizer Inc., New York, N.Y.; an irreversible, steroidal aromatase inactivator used in the treatment of breast cancer); FEMARA® (letrozole, Novartis Pharmaceuticals, East Hanover, N.J.; a nonsteroidal aromatase inhibitor approved by the FDA to treat breast cancer); and NOLVADEX® (tamoxifen, AstraZeneca Pharmaceuticals, LP; a nonsteroidal antiestrogen approved by the FDA to treat breast cancer). Other biologics with which the binding molecules of the invention may be combined include: AVASTIN® (bevacizumab, Genentech Inc.; the first FDA-approved therapy designed to inhibit angiogenesis); and ZEVALIN® (ibritumomab tiuxetan, Biogen Idec, Cambridge, Mass.; a radiolabeled monoclonal antibody currently approved for the treatment of B-cell lymphomas).

In addition, the FDA has approved the following biologics for the treatment of colorectal cancer: AVASTIN®; ERBITUX® (cetuximab, ImClone Systems Inc., New York, N.Y., and Bristol-Myers Squibb, New York, N.Y.; is a monoclonal antibody directed against the epidermal growth factor receptor (EGFR)); GLEEVEC® (imatinib mesylate; a protein kinase inhibitor); and ERGAMISOL® (levamisole hydrochloride, Janssen Pharmaceutica Products, LP, Titusville, N.J.; an immunomodulator approved by the FDA in 1990 as an adjuvant treatment in combination with 5-fluorouracil after surgical resection in patients with Dukes' Stage C colon cancer).

For the treatment of lung cancer, exemplary biologics include TARCEVA® (erlotinib HCL, OSI Pharmaceuticals Inc., Melville, N.Y.; a small molecule designed to target the human epidermal growth factor receptor 1 (HER1) pathway).

For the treatment of multiple myeloma, exemplary biologics include VELCADE® Velcade (bortezomib, Millennium Pharmaceuticals, Cambridge Mass.; a proteasome inhibitor). Additional biologics include THALIDOMID® (thalidomide, Clegene Corporation, Warren, N.J.; an immunomodulatory agent and appears to have multiple actions, including the ability to inhibit the growth and survival of myeloma cells and anti-angiogenesis).

Additional exemplary cancer therapeutic antibodies include, but are not limited to, 3F8, abagovomab, adecatumumab, afutuzumab, alacizumab pegol, alemtuzumab (CAMPATH®, MABCAMPATH®), altumomab pentetate (HYBRI-CEAKER®), anatumomab mafenatox, anrukinzumab (IMA-638), apolizumab, arcitumomab (CEA-SCAN®), bavituximab, bectumomab (LYMPHOSCAN®), belimumab (BENLYSTA®, LYMPHOSTAT-B®), besilesomab (SCINTIMUN®), bevacizumab (AVASTIN®), bivatuzumab mertansine, blinatumomab, brentuximab vedotin, cantuzumab mertansine, capromab pendetide (PROSTASCINT®), catumaxomab (REMOVAB®), CC49, cetuximab (C225, ERBITUX®), citatuzumab bogatox, cixutumumab, clivatuzumab tetraxetan, conatumumab, dacetuzumab, denosumab (PROLIA®), detumomab, ecromeximab, edrecolomab (PANOREX®), elotuzumab, epitumomab cituxetan, epratuzumab, ertumaxomab (REXOMUN®), etaracizumab, farletuzumab, figitumumab, fresolimumab, galiximab, gemtuzumab ozogamicin (MYLOTARG®), girentuximab, glembatumumab vedotin, ibritumomab (ibritumomab tiuxetan, ZEVALIN®), igovomab (INDIMACIS-125®), intetumumab, inotuzumab ozogamicin, ipilimumab, iratumumab, labetuzumab (CEA-CIDE®), lexatumumab, lintuzumab, lucatumumab, lumiliximab, mapatumumab, matuzumab, milatuzumab, minretumomab, mitumomab, nacolomab tafenatox, naptumomab estafenatox, necitumumab, nimotuzumab (THERACIM®, THERALOC®), nofetumomab merpentan (VERLUMA®), ofatumumab (ARZERRA®), olaratumab, oportuzumab monatox, oregovomab (OVAREX®), panitumumab (VECTIBIX®), pentumomab (THERAGYN®), pertuzumab (OMNITARG®), pintumomab, pritumumab, ramucirumab, ranibizumab (LUCENTIS®), rilotumumab, rituximab (MABTHERA®, RITUXAN®), robatumumab, satumomab pendetide, sibrotuzumab, siltuximab, sontuzumab, tacatuzumab tetraxetan (AFP-CIDE®), taplitumomab paptox, tenatumomab, TGN1412, ticilimumab (tremelimumab), tigatuzumab, TNX-650, tositumomab (BEXXAR®), trastuzumab (HERCEPTIN®), tremelimumab, tucotuzumab celmoleukin, veltuzumab, volociximab, votumumab (HUMASPECT®), zalutumumab (HUMAX-EGFR®), and zanolimumab (HUMAX-CD4®).

In other embodiments, the multispecific molecule is administered in combination with a viral cancer therapeutic agent. Exemplary viral cancer therapeutic agents include, but not limited to, vaccinia virus (vvDD-CDSR), carcinoembryonic antigen-expressing measles virus, recombinant vaccinia virus (TK-deletion plus GM-CSF), Seneca Valley virus-001, Newcastle

virus, coxsackie virus A21, GL-ONC1, EBNA1 C-terminal/LMP2 chimeric protein-expressing recombinant modified vaccinia Ankara vaccine, carcinoembryonic antigen-expressing measles virus, G207 oncolytic virus, modified vaccinia virus Ankara vaccine expressing p53, OncoVEX GM-CSF modified herpes-simplex 1 virus, fowlpox virus vaccine vector, recombinant vaccinia prostate-specific antigen vaccine, human papillomavirus 16/18 L1 virus-like particle/AS04 vaccine, MVA-EBNA1/LMP2 Inj. vaccine, quadrivalent HPV vaccine, quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine (GARDASIL®), recombinant fowlpox-CEA(6D)/TRICOM vaccine; recombinant vaccinia-CEA(6D)-TRICOM vaccine, recombinant modified vaccinia Ankara-5T4 vaccine, recombinant fowlpox-TRICOM vaccine, oncolytic herpes virus NV1020, HPV L1 VLP vaccine V504, human papillomavirus bivalent (types 16 and 18) vaccine (CERVARIX®), herpes simplex virus HF10, Ad5CMV-p53 gene, recombinant vaccinia DF3/MUC1 vaccine, recombinant vaccinia-MUC-1 vaccine, recombinant vaccinia-TRICOM vaccine, ALVAC MART-1 vaccine, replication-defective herpes simplex virus type I (HSV-1) vector expressing human Preproenkephalin (NP2), wild-type reovirus, reovirus type 3 Dearing (REOLYSIN®), oncolytic virus HSV1716, recombinant modified vaccinia Ankara (MVA)-based vaccine encoding Epstein-Barr virus target antigens, recombinant fowlpox-prostate specific antigen vaccine, recombinant vaccinia prostate-specific antigen vaccine, recombinant vaccinia-B7.1 vaccine, rAd-p53 gene, Ad5-delta24RGD, HPV vaccine 580299, JX-594 (thymidine kinase-deleted vaccinia virus plus GM-CSF), HPV-16/18 L1/AS04, fowlpox virus vaccine vector, vaccinia-tyrosinase vaccine, MEDI-517 HPV-16/18 VLP AS04 vaccine, adenoviral vector containing the thymidine kinase of herpes simplex virus TK99UN, HspE7, FP253/Fludarabine, ALVAC(2) melanoma multi-antigen therapeutic vaccine, ALVAC-hB7.1, canarypox-hIL-12 melanoma vaccine, Ad-REIC/Dkk-3, rAd-IFN SCH 721015, TIL-Ad-INFg, Ad-ISF35, and coxsackievirus A21 (CVA21, CAVATAK®).

In other embodiments, the multispecific molecule is administered in combination with a nanopharmaceutical. Exemplary cancer nanopharmaceuticals include, but not limited to, ABRAXANE® (paclitaxel bound albumin nanoparticles), CRLX101 (CPT conjugated to a linear cyclodextrin-based polymer), CRLX288 (conjugating docetaxel to the biodegradable polymer poly (lactic-co-glycolic acid)), cytarabine liposomal (liposomal Ara-C, DEPOCYT™), daunorubicin liposomal (DAUNOXOME®), doxorubicin liposomal (DOXIL®, CAELYX®),

encapsulated-daunorubicin citrate liposome (DAUNOXOME®), and PEG anti-VEGF aptamer (MACUGEN®).

In some embodiments, the multispecific molecule is administered in combination with paclitaxel or a paclitaxel formulation, e.g., TAXOL®, protein-bound paclitaxel (e.g., ABRAXANE®). Exemplary paclitaxel formulations include, but are not limited to, nanoparticle albumin-bound paclitaxel (ABRAXANE®, marketed by Abraxis Bioscience), docosahexaenoic acid bound-paclitaxel (DHA-paclitaxel, Taxoprexin, marketed by Protarga), polyglutamate bound-paclitaxel (PG-paclitaxel, paclitaxel poliglumex, CT-2103, XYOTAX, marketed by Cell Therapeutic), the tumor-activated prodrug (TAP), ANG105 (Angiopep-2 bound to three molecules of paclitaxel, marketed by ImmunoGen), paclitaxel-EC-1 (paclitaxel bound to the erbB2-recognizing peptide EC-1; see Li *et al.*, *Biopolymers* (2007) 87:225-230), and glucose-conjugated paclitaxel (e.g., 2'-paclitaxel methyl 2-glucopyranosyl succinate, see Liu *et al.*, *Bioorganic & Medicinal Chemistry Letters* (2007) 17:617-620).

Exemplary RNAi and antisense RNA agents for treating cancer include, but not limited to, CALAA-01, siG12D LODER (Local Drug EluteR), and ALN-VSP02.

Other cancer therapeutic agents include, but not limited to, cytokines (e.g., aldesleukin (IL-2, Interleukin-2, PROLEUKIN®), alpha Interferon (IFN-alpha, Interferon alfa, INTRON® A (Interferon alfa-2b), ROFERON-A® (Interferon alfa-2a)), Epoetin alfa (PROCRIT®), filgrastim (G-CSF, Granulocyte - Colony Stimulating Factor, NEUPOGEN®), GM-CSF (Granulocyte Macrophage Colony Stimulating Factor, sargramostim, LEUKINE™), IL-11 (Interleukin-11, oprelvekin, NEUMEGA®), Interferon alfa-2b (PEG conjugate) (PEG interferon, PEG-INTRON™), and pegfilgrastim (NEULASTA™)), hormone therapy agents (e.g., aminoglutethimide (CYTADREN®), anastrozole (ARIMIDEX®), bicalutamide (CASODEX®), exemestane (AROMASIN®), fluoxymesterone (HALOTESTIN®), flutamide (EULEXIN®), fulvestrant (FASLODEX®), goserelin (ZOLADEX®), letrozole (FEMARA®), leuprolide (ELIGARD™, LUPRON®, LUPRON DEPOT®, VIADUR™), megestrol (megestrol acetate, MEGACE®), nilutamide (ANANDRON®, NILANDRON®), octreotide (octreotide acetate, SANDOSTATIN®, SANDOSTATIN LAR®), raloxifene (EVISTA®), romiplostim (NPLATE®), tamoxifen (NOVALDEX®), and toremifene (FARESTON®)), phospholipase A2 inhibitors (e.g., anagrelide (AGRYLIN®)), biologic response modifiers (e.g., BCG (THERACYS®, TICE®), and Darbepoetin alfa (ARANESP®)), target therapy agents (e.g.,

bortezomib (VELCADE®), dasatinib (SPRYCEL™), denileukin diftitox (ONTAK®), erlotinib (TARCEVA®), everolimus (AFINITOR®), gefitinib (IRESSA®), imatinib mesylate (STI-571, GLEEVEC™), lapatinib (TYKERB®), sorafenib (NEXAVAR®), and SU11248 (sunitinib, SUTENT®)), immunomodulatory and antiangiogenic agents (e.g., CC-5013 (lenalidomide, REVLIMID®), and thalidomide (THALOMID®)), glucocorticosteroids (e.g., cortisone (hydrocortisone, hydrocortisone sodium phosphate, hydrocortisone sodium succinate, ALA-CORT®, HYDROCORT ACETATE®, hydrocortone phosphate LANACORT®, SOLU-CORTEF®), decadron (dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, DEXASONE®, DIODEX®, HEXADROL®, MAXIDEX®), methylprednisolone (6-methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, DURALONE®, MEDRALONE®, MEDROL®, M-PREDNISOL®, SOLU-MEDROL®), prednisolone (DELTA-CORTEF®, ORAPRED®, PEDIAPRED®, PRELONE®), and prednisone (DELTASONE®, LIQUID PRED®, METICORTEN®, ORASONE®)), and bisphosphonates (e.g., pamidronate (AREDIA®), and zoledronic acid (ZOMETA®))

In some embodiments, the multispecific molecule is used in combination with a tyrosine kinase inhibitor (e.g., a receptor tyrosine kinase (RTK) inhibitor). Exemplary tyrosine kinase inhibitor include, but are not limited to, an epidermal growth factor (EGF) pathway inhibitor (e.g., an epidermal growth factor receptor (EGFR) inhibitor), a vascular endothelial growth factor (VEGF) pathway inhibitor (e.g., an antibody against VEGF, a VEGF trap, a vascular endothelial growth factor receptor (VEGFR) inhibitor (e.g., a VEGFR-1 inhibitor, a VEGFR-2 inhibitor, a VEGFR-3 inhibitor)), a platelet derived growth factor (PDGF) pathway inhibitor (e.g., a platelet derived growth factor receptor (PDGFR) inhibitor (e.g., a PDGFR- β inhibitor)), a RAF-1 inhibitor, a KIT inhibitor and a RET inhibitor. In some embodiments, the anti-cancer agent used in combination with the multispecific molecule is selected from the group consisting of: axitinib (AG013736), bosutinib (SKI-606), cediranib (RECENTIN™, AZD2171), dasatinib (SPRYCEL®, BMS-354825), erlotinib (TARCEVA®), gefitinib (IRESSA®), imatinib (Gleevec®, CGP57148B, STI-571), lapatinib (TYKERB®, TYVERB®), lestaurtinib (CEP-701), neratinib (HKI-272), nilotinib (TASIGNA®), semaxanib (semaxinib, SU5416), sunitinib (SUTENT®, SU11248), toceranib (PALLADIA®), vandetanib (ZACTIMA®, ZD6474), vatalanib (PTK787, PTK/ZK), trastuzumab (HERCEPTIN®), bevacizumab (AVASTIN®), rituximab (RITUXAN®), cetuximab (ERBITUX®), panitumumab (VECTIBIX®), ranibizumab

(Lucentis®), nilotinib (TASIGNA®), sorafenib (NEXAVAR®), alemtuzumab (CAMPATH®), gemtuzumab ozogamicin (MYLOTARG®), ENMD-2076, PCI-32765, AC220, dovitinib lactate (TKI258, CHIR-258), BIBW 2992 (TOVOK™), SGX523, PF-04217903, PF-02341066, PF-299804, BMS-777607, ABT-869, MP470, BIBF 1120 (VARGATEF®), AP24534, JNJ-26483327, MGCD265, DCC-2036, BMS-690154, CEP-11981, tivozanib (AV-951), OSI-930, MM-121, XL-184, XL-647, XL228, AEE788, AG-490, AST-6, BMS-599626, CUDC-101, PD153035, pelitinib (EKB-569), vandetanib (zactima), WZ3146, WZ4002, WZ8040, ABT-869 (linifanib), AEE788, AP24534 (ponatinib), AV-951(tivozanib), axitinib, BAY 73-4506 (regorafenib), brivanib alaninate (BMS-582664), brivanib (BMS-540215), cediranib (AZD2171), CHIR-258 (dovitinib), CP 673451, CYC116, E7080, Ki8751, masitinib (AB1010), MGCD-265, motesanib diphosphate (AMG-706), MP-470, OSI-930, Pazopanib Hydrochloride, PD173074, nSorafenib Tosylate (Bay 43-9006), SU 5402, TSU-68 (SU6668), vatalanib, XL880 (GSK1363089, EXEL-2880). Selected tyrosine kinase inhibitors are chosen from sunitinib, erlotinib, gefitinib, or sorafenib. In one embodiment, the tyrosine kinase inhibitor is sunitinib.

In one embodiment, the multispecific molecule is administered in combination with one or more of: an anti-angiogenic agent, or a vascular targeting agent or a vascular disrupting agent. Exemplary anti-angiogenic agents include, but are not limited to, VEGF inhibitors (*e.g.*, anti-VEGF antibodies (*e.g.*, bevacizumab); VEGF receptor inhibitors (*e.g.*, itraconazole); inhibitors of cell proliferation and/or migration of endothelial cells (*e.g.*, carboxyamidotriazole, TNP-470); inhibitors of angiogenesis stimulators (*e.g.*, suramin), among others. A vascular-targeting agent (VTA) or vascular disrupting agent (VDA) is designed to damage the vasculature (blood vessels) of cancer tumors causing central necrosis (reviewed in, *e.g.*, Thorpe, P.E. (2004) *Clin. Cancer Res.* Vol. 10:415-427). VTAs can be small-molecule. Exemplary small-molecule VTAs include, but are not limited to, microtubule destabilizing drugs (*e.g.*, combretastatin A-4 disodium phosphate (CA4P), ZD6126, AVE8062, Oxi 4503); and vadimezan (ASA404).

Immune checkpoint inhibitors

In other embodiments, methods described herein comprise use of an immune checkpoint inhibitor in combination with the multispecific molecule. The methods can be used in a therapeutic protocol *in vivo*.

In embodiments, an immune checkpoint inhibitor inhibits a checkpoint molecule. Exemplary checkpoint molecules include but are not limited to CTLA4, PD1, PD-L1, PD-L2, TIM3, LAG3, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), BTLA, KIR, MHC class I, MHC class II, GAL9, VISTA, BTLA, TIGIT, LAIR1, and A2aR. See, e.g., Pardoll. *Nat. Rev. Cancer* 12.4(2012):252-64, incorporated herein by reference.

In embodiments, the immune checkpoint inhibitor is a PD-1 inhibitor, e.g., an anti-PD-1 antibody such as Nivolumab, Pembrolizumab or Pidilizumab. Nivolumab (also called MDX-1106, MDX-1106-04, ONO-4538, or BMS-936558) is a fully human IgG4 monoclonal antibody that specifically inhibits PD1. See, e.g., US 8,008,449 and WO2006/121168. Pembrolizumab (also called Lambrolizumab, MK-3475, MK03475, SCH-900475 or KEYTRUDA®; Merck) is a humanized IgG4 monoclonal antibody that binds to PD-1. See, e.g., Hamid, O. *et al.* (2013) *New England Journal of Medicine* 369 (2): 134–44, US 8,354,509 and WO2009/114335. Pidilizumab (also called CT-011 or Cure Tech) is a humanized IgG1k monoclonal antibody that binds to PD1. See, e.g., WO2009/101611. In one embodiment, the inhibitor of PD-1 is an antibody molecule having a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence of Nivolumab, Pembrolizumab or Pidilizumab. Additional anti-PD1 antibodies, e.g., AMP 514 (Amplimmune), are described, e.g., in US 8,609,089, US 2010028330, and/or US 20120114649.

In some embodiments, the PD-1 inhibitor is an immunoadhesin, e.g., an immunoadhesin comprising an extracellular/PD-1 binding portion of a PD-1 ligand (e.g., PD-L1 or PD-L2) that is fused to a constant region (e.g., an Fc region of a heavy chain). In embodiments, the PD-1 inhibitor is AMP-224 (B7-DCIg, e.g., described in WO2011/066342 and WO2010/027827), a PD-L2 Fc fusion soluble receptor that blocks the interaction between B7-H1 and PD-1.

In embodiments, the immune checkpoint inhibitor is a PD-L1 inhibitor, e.g., an antibody molecule. In some embodiments, the PD-L1 inhibitor is YW243.55.S70, MPDL3280A, MEDI-4736, MSB-0010718C, or MDX-1105. In some embodiments, the anti-PD-L1 antibody is MSB0010718C (also called A09-246-2; Merck Serono), which is a monoclonal antibody that binds to PD-L1. Exemplary humanized anti-PD-L1 antibodies are described, e.g., in WO2013/079174. In one embodiment, the PD-L1 inhibitor is an anti-PD-L1 antibody, e.g., YW243.55.S70. The YW243.55.S70 antibody is described, e.g., in WO 2010/077634. In one

embodiment, the PD-L1 inhibitor is MDX-1105 (also called BMS-936559), which is described, e.g., in WO2007/005874. In one embodiment, the PD-L1 inhibitor is MDPL3280A (Genentech / Roche), which is a human Fc-optimized IgG1 monoclonal antibody against PD-L1. See, e.g., U.S. Patent No.: 7,943,743 and U.S. Publication No.: 20120039906. In one embodiment, the inhibitor of PD-L1 is an antibody molecule having a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence of YW243.55.S70, MPDL3280A, MEDI-4736, MSB-0010718C, or MDX-1105.

In embodiments, the immune checkpoint inhibitor is a PD-L2 inhibitor, e.g., AMP-224 (which is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD1 and B7-H1. See, e.g., WO2010/027827 and WO2011/066342.

In one embodiment, the immune checkpoint inhibitor is a LAG-3 inhibitor, e.g., an anti LAG-3 antibody molecule. In embodiments, the anti-LAG-3 antibody is BMS-986016 (also called BMS986016; Bristol-Myers Squibb). BMS-986016 and other humanized anti-LAG-3 antibodies are described, e.g., in US 2011/0150892, WO2010/019570, and WO2014/008218.

In embodiments, the immune checkpoint inhibitor is a TIM-3 inhibitor, e.g., anti-TIM3 antibody molecule, e.g., described in U.S. Patent No.: 8,552,156, WO 2011/155607, EP 2581113 and U.S. Publication No.: 2014/044728.

In embodiments, the immune checkpoint inhibitor is a CTLA-4 inhibitor, e.g., anti-CTLA-4 antibody molecule. Exemplary anti-CTLA4 antibodies include Tremelimumab (IgG2 monoclonal antibody from Pfizer, formerly known as ticilimumab, CP-675,206); and Ipilimumab (also called MDX-010, CAS No. 477202-00-9). Other exemplary anti-CTLA-4 antibodies are described, e.g., in U.S. Pat. No. 5,811,097.

EXAMPLES

The following examples are intended to be illustrative, and are not meant in any way to be limiting.

Example 1. Generation of multiple α CCR2/ α CSF1R bispecific antibody molecules

1. Construction of the plasmids.

The DNA encoding the protein sequences was optimized for expression in *Cricetulus griseus*, synthesized, and cloned into the pcDNA3.4-TOPO (Life Technologies A14697) using

Gateway cloning. All constructs contained an Ig Kappa leader sequence (ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAGGATCTACAGGA (SEQ ID NO: 115), METDTLLLWVLLLWVPGSTG (SEQ ID NO: 116)). The nucleic acid sequences used are shown in Table 1.

Table 1: Exemplary nucleic acid sequences of antibodies

SEQ ID NO	Description	Nucleic Acid Sequence
SEQ ID NO: 1	α CCR2 MC12 VH	CAGGTCCAGCTGCAAGAGTCTGGCCCTGGACTGGTTCAG CCCTCTCAGACCCTGTCTCTGACCTGTACCGTGTCCGGCT TCTCCCTGACCGACTTCTCTGTGCACTGGGTCCGACAGCC TCCAGGCAAAGGACTGGAATGGATGGGCAGAATCAGATC CGAGGGCAACACCGACTACAACAGCGCCCTGAAGTCCCG GCTGTCTATCAGCAGAGACACCTCCAAGAGCCAGGTGTT CCTGAAGATGAACTCCCTGCAGACCGAGGACACCGCCAT CTATTTCTGCACCAGAGGCGACATCCTCGGCTTCGGCTAT TGGGGACAGGGCGTGATGGTCAACCGTTAGCTCT
SEQ ID NO: 2	α CCR2 MC12 VL	GACATCGTGATGACCCAGTCTCCACTGTCCGTGTCTGTGA CCCCTGGCGAGTCTGCCTCCATCTCCTGCAGATCCTCCAA GAGCCTGCTGCACTTCAAGGGCATCACCTTCGTGTACTGG TATCTGCAGAAGCCCGGCCAGTCTCCTCAGCTGCTGATCT TCAGAATGTCCAGCCTGGCCTCTGGCGTGCCCGATAGATT TTCTGGCTCCGGCTCCGAGACAGACTTCACCCTGAAGATC TCCAGAGTGGAAGCCGAGGACGTGGGACACCTACTATTGT GGCCAGCTGCTGGAAAACCCCTACACCTTTGGCGCTGGC ACCAAGCTGGAAGTGAAG
SEQ ID NO: 3	R2b CH1	GCTCAGACCACCGCTCCTAGCGTGTACCCTTTGGCTCCTG GCTGTGGCGACACCACCTCTTCTACAGTGACCCTGGGCTG TCTGGTCAAGGGCTACTTTCCTGAGCCTGTGACCGTGACC TGGAAGTCTGGTGCCCTGTCCTCCGACGTGCACACCTTTC CAGCTGTGCTGCAGTCCGGCCTGTACACCCTGACATCCTC CGTGACCTCTTCCACCTGGCCTAGCCAGACCGTGACATGC AATGTGGCTCACCTGCCTCCAGCACCAAGGTGGACAAG AAGGTGGAACGGCGG
SEQ ID NO: 4	R2b CL	AGAGCTGACGCTGCCCCCTACCGTGTCTATCTTCCCTCCAT CCATGGAACAGCTGACCTCTGGCGGAGCTACCGTCGTGT GCTTCGTGAACAACCTTCTACCCTCGGGACATCTCCGTGAA GTGGAAGATCGACGGCTCTGAGCAGCGAGATGGCGTGCT GGATTCTGTGACCGACCAGGACTCCAAGGACAGCACCTA CTCCATGTCTAGCACCTGAGCCTGACCAAGGTGGAATA CGAGCGGCACAACCTGTATACCTGCGAGGTGGTGCAAA GACCTCCAGCTCTCCCGTGGTCAAGTCCTTCAACCGGAAC GAGTGC
SEQ ID NO: 5	α mCSF1R VH	CAGGTCCAGTTGCAGCAGTCTGGCGCTGAGCTGGTCAAG CCTGGATCCTCCGTGAAGATCTCCTGCAAGGCCTCCGGCT ACACCTTCACCTCCAACCTCATGCACTGGATCAAGCAGCA

		GCCCCGGCAACGGCCTGGAATGGATCGGATGGATCTATCC TGGCGACGGCGACACCGAGTACAACCAGAAGTTCAACGG CAAGGCTACCCTGACCGCCGACAAGTCCTCTTCCACCGCT TACATGCAGCTGTCCAGCCTGACCTCTGAGGACTCCGCCG TGTACTTCTGCGCCGTGAATTATGGCGGCTACGTGCTGGA TGCTTGGGGCCAAGGCGCTTCTGTGACAGTGTCTCT
SEQ ID NO: 6	R2a CH1	GCCGAGACAACCGCTCCTAGCGTTTACCCTCTGGCTCCTG GCACAGCCCTGAAGTCCAACCTCTATGGTCACCCTGGGCT GCCTGGTCAAGGGCTACTTTCCTGAGCCTGTGACCGTGAC CTGGAACCTCTGGTGCTCTGTCTAGCGGCGTGCACACCTTT CCAGCTGTGCTGCAGAGCGGCCTGTACACCCTGACATCT AGCGTGACCGTGCCTTCCAGCACCTGGTCTAGTCAGGCTG TGACCTGCAACGTGGCCCATCCTGCCTCTTCTACCAAGGT GGACAAGAAAATCGTGCCCAGAGAGTGCAAC
SEQ ID NO: 7	α mCSF1R VL	GAGATCGTGCTGACCCAGTCTCCTACCACCATGGCTGCTA GCCCTGGCGAGAAAGTGACAATTACCTGCCGGGGCCTCCT CCTCCACCAACTACATGTCCTGGTATCAGCAGAAGTCCG GCGCCTCTCCTAAGCCTTGGATCTACGAGACATCCAAGCT GGCCTCTGGCGTGCCCGATAGATTTTCCGGCTCTGGCTCC GGCACCTCCTACAGCTTCACCATCTCCAGCATGGAAACA GAGGACGCCGCCACCTACTACTGCCACCAGTGGTCATCT ACCCCTCTGACCTTTGGCAGCGGCACCAAGCTGGAAATC AAG
SEQ ID NO: 8	R2a CL	AGAGCTGACGCCGCTCCTACCGTGTCTATCTTCCCTCCAT CCATGGAACAGCTGACCTCCGGCGGAGCTACCGTCGTGT GTTTCGTGAACAACCTTCTACCCTCGGGACATCTCCGTGAA GTGGAAGATCGACGGCTCTGAGCAGCGAGATGGCGTGCT GGATTCTGTGACCGACCAGGACTCCAAGGACAGCACCTA CTCCATGTCTAGCACCCCTGAGCCTGACCAAGGTGGAATA CGAGCGGCACAACCTGTATACCTGCGAGGTGGTGCAACA GACCTCCAGCTCTCCCGTGGTCAAGTCCTTCAACCGGAAC GAGTGC
SEQ ID NO: 9	mFc Knob	ACCATTAAGCCTTGTCTCCATGCAAGTGCCCCGCTCCTA ATCTGCTCGGAGGGCCCTTCCGTGTTTCATCTTTCCACCTAA GATCAAGGACGTGCTGATGATCTCCCTGTCTCCTATCGTG ACCTGCGTGGTGGTGGACGTGTCCGAGGATGATCCTGAC GTGCAGATCAGTTGGTTCGTGAACAACGTGGAAGTGCAC ACCGCTCAGACCCAGACACACAGAGAGGACTACAACCTCT ACCCTGAGAGTGGTGTCTGCCCTGCCTATCCAGCATCAGG ACTGGATGTCCGGCAAAGAATTCAAGTGCAAAGTGAACA ACAAGGACCTGCCTGCTCCAATCGAGCGGACCATCTCTA AGCCTAAGGGCTCTGTCAAGGGCCCCTCAGGTGTACGTTCT GCCTCCTTGCGAGGAAGAGATGACCAAGAAACAAGTGAC ACTGTGGTGCATGGTCACAGACTTCATGCCCCGAGGACAT CTACGTGGAATGGACCAACAACGGCAAGACCGAGCTGAA CTACAAGAACACCGAGCCTGTGCTGGACTCCGACGGCTC CTACTTCATGTACTCCAAGCTGCGCGTCGAGAAGAAGAA CTGGGTGCGAGAGAACTCCTACTCCTGCTCCGTGGTGCAC GAGGGCCTGCACAATCACCACACCACCAAGTCCTTCTCTC GGACCCCTGGCAAG

SEQ ID NO: 10	mFc Hole	ACCATCAAGCCCTGTCCTCCATGCAAGTGCCCCGCTCCTA ATCTGCTCGGAGGCCCTTCCGTGTTTCATCTTCCCACCTAA GATCAAGGACGTGCTGATGATCTCCCTGTCTCCTATCGTG ACCTGCGTGGTGGTGGACGTGTCCGAGGATGATCCTGAC GTGCAGATCAGTTGGTTCGTGAACAACGTGGAAGTGCAC ACCGCTCAGACCCAGACACACAGAGAGGACTACAACAGC ACCCTGAGAGTGGTGTCTGCCCTGCCAATCCAGCACCAG GATTGGATGTCCGGCAAAGAATTCAAGTGCAAAGTGAAC AACAAGGACCTGCCTGCTCCAATCGAGCGGACCATCTCT AAGCCTAAGGGCTCTGTGCGGGCTCCCCAAGTTTGTGTTC TGCCTCCACCTGAGGAAGAGATGACCAAGAAACAAGTGA CCCTGTCTTGTGCCGTGACCGACTTCATGCCCCGAGGACAT CTACGTGGAATGGACCAACAATGGCAAGACCGAGCTGAA CTACAAGAACACCGAGCCTGTGCTGGACTCCGACGGCTC CTACTTCATGGTGTCTAAGCTGCGCGTCGAGAAGAAGAA CTGGGTCGAGAGAAACTCCTACTCCTGCTCCGTGGTGCAC GAGGGCCTGCACAATCACCACACCACCAAGTCCTTCTCTC GGACCCCTGGCAAG
SEQ ID NO: 11	αhCCR2 plozalizumab VH	GAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTAAG CCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCTTCTGGCT TCACCTTCTCCGCCTACGCCATGAACTGGGTCCGACAGGC TCCTGGCAAAGGCCTGGAATGGGTTCGGAAGAATCCGGAC CAAGAACAACAACCTACGCCACCTACTACGCCGACTCCGT GAAGGACCGGTTACCATCTCTCGGGACGACTCCAAGAA CACCCTGTACCTGCAGATGAACTCCCTGAAAACCGAGGA CACCGCCGTGTACTACTGCACCACCTTCTACGGCAATGGC GTGTGGGGACAGGGCACACTGGTTACCGTTTCTTCCGCCT CCACCAAGGGACCCTCTGTGTTTCCTCTGGCTCCCTCCAG CAAGTCTACCTCTGGTGGAACAGCTGCCCTGGGCTGCCTG GTCAAGGATTACTTTCCTGAGCCTGTGACCGTGTCTCTGG
SEQ ID NO: 12	hCH1	GCTTCTACCAAGGGACCCAGCGTGTTCCCTCTGGCTCCTT CCAGCAAGTCTACCTCTGGCGGAACAGCTGCTCTGGGCT GCCTGGTCAAGGACTACTTTCCTGAGCCTGTGACCGTGTCT TTGGAACCTCTGGCGCTCTGACATCCGGCGTGCACACATTT CCAGCTGTGCTGCAGTCCTCCGGCCTGTACTCTCTGTCTCT CTGTCGTGACCGTGCCTTCCAGCTCTCTGGGAACCCAGAC CTACATCTGCAATGTGAACCACAAGCCTTCCAACACCAA GGTGGACAAGAGAGTGGAACCCAAGTCCTGC
SEQ ID NO: 13	hFc Knob	GATAAGACCCACACATGTCCTCCATGCCCTGCCCCCTGAGC TGCTGGGCGGACCTTCCGTGTTCCCTGTTCCCTCCAAAGCC CAAGGACACCCTGATGATCAGCCGGACCCCTGAAGTGAC CTGCGTGGTGGTGGATGTGTCCACGAGGATCCCGAAGT GAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAA CGCCAAGACCAAGCCCAGAGAGGAACAGTACAACAGCA CCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCATCAGGA CTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAA CAAGGCCCTGCCTGCCCCCTATCGAGAAAACCATCAGCAA GGCCAAGGGCCAGCCCCGCGAACCTCAGGTGTACACACT GCCTCCCTGCCGGGAAGAGATGACCAAGAACCAGGTGTCT CCTGTGGTGCCTGGTCAAGGGCTTCTACCCCTCCGATATC GCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAA

		CTACAAGACCACCCCTCCCGTGCTGGACAGCGACGGCAG CTTCTTCCTGTACTCCAAACTGACCGTGGACAAGAGCCGG TGGCAGCAGGGCAATGTGTTTCAGCTGTAGCGTGATGCAC GAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGC CTGTCTCCTGGCAAA
SEQ ID NO: 14	α hCCR2 plozalizumab VL	GACGTGGTCATGACACAGAGCCCTCTGTCTCTGCCCCGTGA CATTGGGACAGCCTGCCTCCATCTCCTGCAAGTCCTCTCA GTCCCTGCTGGACTCTGACGGCAAGACCTTCCTGAACTGG TTCCAGCAGCGGCCTGGCCAGTCTCCTAGAAGGCTGATCT ACCTGGTGTCCAAGCTGGATTCTGGCGTGCCCGACAGATT CTCCGGCTCTGGCTCTGGCACCGACTTCACCCTGAAGATC TCCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGT TGGCAGGGCACCCACTTTCCATACACCTTCGGCCAGGGC ACCAGACTGGAAATCAAG
SEQ ID NO: 15	hCL (kappa)	AGAACAGTGGCCGCTCCTTCCGTGTTTCATCTTCCCACCCT CCGACGAGCAGCTGAAGTCCGGCACCGCTTCTGTCGTGT GCCTGCTCAACAACCTTCTACCCTCGGGAAGCCAAGGTGC AGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAACTCCC AGGAATCCGTCACCGAGCAGGACTCCAAGGACAGCACCT ACTCCCTGTCCTCCACCCTGACCCTGTCCAAGGCCGACTA CGAGAAGCACAAAGGTGTACGCCTGCGAAGTGACCCACCA GGGCCTGAGCAGCCCCGTGACCAAGTCCTTCAACCGGGG CGAGTGC
SEQ ID NO: 16	α hCCR2 D1 VH	GAAGTGCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAAA CCTGGCGCCTCTGTGAAGGTGTCCTGCAAGGCTTCTGGCT ACACCTTTACCGGCTACCACATGCACTGGGTCCGACAGG CTCCAGGACAAGGCTTGGAATGGATGGGCTGGATCAACC CCAACCTCCGGCGTGACCAAATACGCCCAGAAATTCCAGG GCAGAGTGACCATGACCAGAGACACCTCCATCAACACCG CCTACATGGAAGTGTCCCGGCTGAGATTTCGACGACACCG ACGTGTACTACTGTGCCACCGGCGGCTTTGGCTATTGGGG AGAGGGAACACTGGTCAACCGTGTCTCTCC
SEQ ID NO: 17	α hCCR2 D1 VL	CTGCCCCGTGTTGACCCAGCCTCCTAGCGTTTCCAAGGGCC TGAGACAGACCGCCACACTGACCTGTACCGGCAACTCTA ACAACGTGGGCAATCAGGGCGCTGCCTGGTTGCAGCAGC ATCAGGGACAGCCTCCAAAGCTGCTGTCCTACCGGAACC ACAACAGACCTAGCGGCGTGTCCGAGCGGTTTCAGCCCTT CTAGATCTGGCGACACCTCCAGCCTGACCATCACTGGACT GCAGCCTGAGGACGAGGCCGACTACTATTGTCTGGCCTG GGACAGCTCCCTGCGGGCCTTTGTTTTTGGCACCGGCACC AAGCTGACCGTGCTG
SEQ ID NO: 18	hCL (lambda)	GGACAACCTAAGGCCAATCCTACCGTGACACTGTTCCCTC CATCCTCCGAGGAACTGCAGGCCAACAAGGCTACCCTCG TGTGCCTGATCTCCGACTTTTACCCTGGCGCTGTGACCGT GGCCTGGAAGGCTGATGGATCTCCTGTGAAGGCTGGCGT GGAAACCACCAAGCCTTCCAAGCAGTCCAACAACAAATA CGCCGCCTCCTCCTACCTGTCTCTGACCCCTGAACAGTGG AAGTCCCACCGGTCCTACAGCTGCCAAGTGACCCATGAG GGCTCCACCGTGGAAGAACCGTGGCTCCTACCGAGTGC TCC

SEQ ID NO: 19	α hCCR2 42G7 VH	CAGGTGCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAAA CCTGGCGCCTCTGTGAAGGTGTCCTGCAAGGCTTCCGGCT ACACCTTCTCCAGCTACTACATGCACTGGGTCCGACAGGC CCCTGGACAAGGATTGGAGTGGATGGGCATCATCAACCC CTCTGGCGGCAACACCTCTTACGCCCAGAAATTCCAGGG CAGAGTGACCATGACCAGAGACACCTCCACCAGCACCGT GTACATGGAACTGTCCAGCCTGAGATCCGAGGACACCGC CGTGTACTACTGTGCCAGAGGCGGATAACCAGCTGCCTCA CGGTAGAGCCAGAGCCTTCGATATGTGGGGCCAGGGCAC AATGGTCACCGTGTCTCT
SEQ ID NO: 20	α hCCR2 42G7 VL	GCCATCAGAATGACCCAGTCTCCACTGAGCCTGCCTGTG ACATTGGGCCAGCCTGCCTCTATCTCCTGCACCTCCTCTC AGTCTCTGGTGTACAGAGATGGCACCACCTACCTGAACT GGTTCCAGCAGAGGCCTGGCCAGTCTCCTAGACGGCTGA TCTACAAGGTGTCCAACAGAGACTCTGGCGTGCCCGACA GATTCACCGGCTCTGGCTCTGGCACCACATTCACCCTGAC CATCTCCAGAGTGGAAGCCGAGGACGTGGGCATCTACTA CTGTATGCAGGGCACCCACTGGCCTCTGACCTTTGGCCAG GGAACAAAGGTGGAAATCAAG
SEQ ID NO: 21	α hCCR2 43G12 VH	GAGGTGCAGCTGGTTGAATCTGGCGGAGGATTGGTTCAG CCTGGCGGCTCTCTGAGACTGTCTTGTGTGGCCTCTGGCT TCACCTTCTCCGACTACTGGATGTCCTGGGTCCGACAGGC TCCTGGCAAAGGACTGGAATGGGTTCGCCAACATCAAGAA AGACGGCTCCGTGAACTACTACGTGGACTCCGTGAAGGG CAGATTCACCATCTCTCGGGACAACGCCAAGAACTCCCT GTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGC CGTGTACTACTGCACCAGATTTCGATTACTGGGGCCAGGG CACCTGGTCACAGTGTCTCT
SEQ ID NO: 22	α hCCR2 43G12 VL	CAGGCTGGCTTGACCCAGCCTCCTAGCGTTTCCAAGGGCC TGAGACAGACCGCCACACTGACCTGTACCGGCAACTCTA ACAACGTGGGCAATCAGGGCGCTGCCTGGTTGCAGCAGC ATCAGGGACATCCTCCAAAGCTGCTGTTCTACCGGAACA ACAACAGAGCCTCCGGCATCTCCGAGCGGCTGTCTGCTTC TAGATCCGGCAATACCGCCAGCCTGACCATCACTGGACT GCAGCCTGAGGACGAGGCCGACTACTATTGCCTGACCTG GGACTCCTCTCTGTCCGTGGTGGTTTTTGGCGGAGGCACC AAGCTGACAGTGCTG
SEQ ID NO: 23	α hCSF1R emactuzumab VH	CAGGTGCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAAA CCTGGCGCCTCTGTGAAGGTGTCCTGCAAGGCTTCCGGCT ACACCTTTACCAGCTACGACATCTCCTGGGTCCGACAGGC TCCTGGACAAGGCTTGGAATGGATGGGCGTGATCTGGAC CGATGGCGGCACCAATTACGCCCAGAACTGCAGGGCAG AGTGACCATGACCACCGACACCTCTACCTCCACCGCCTAC ATGGAAGTGCGGTCCCTGAGATCTGACGACACCGCCGTG TACTACTGCGCCAGAGATCAGCGGCTGTACTTCGATGTGT GGGGCCAGGGCACAACCGTGACAGTGTCTCT
SEQ ID NO: 24	α hCSF1R emactuzumab VL	GACATCCAGATGACCCAGTCTCCATCCTCTCTGTCCGCCT CTGTGGGCGACAGAGTGACCATCACCTGTAGAGCCTCCG AGGACGTGAACACCTACGTGTCCTGGTATCAGCAGAAGC CCGGCAAGGCTCCCAAGCTGCTGATCTACGCCGCCTCTA

		ACAGATACACCGGCGTGCCCTCTAGATTCTCCGGCTCTGG CTCTGGCACCGACTTTACCCTGACAATCTCCAGCCTGCAG CCTGAGGACTTCGCCACCTACTACTGCCAGCAGTCCTTCA GCTACCCACCTTTGGCCAGGGCACCAAGCTGGAAATCA AG
SEQ ID NO: 25	hFc Hole	GATAAGACCCACACCTGTCCTCCCTGCCCTGCCCCTGAAC TGCTGGGCGGACCTAGCGTGTTCCCTGTTCCCTCCAAAGCC CAAGGACACCCTGATGATCAGCCGGACCCCTGAAGTGAC CTGCGTGGTGGTGGATGTGTCCCACGAGGATCCCGAAGT GAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAA CGCCAAGACCAAGCCCAGAGAGGAACAGTACAACAGCA CCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGG ACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCA ACAAGGCCCTGCCAGCCCCTATCGAGAAAACCATCAGCA AGGCCAAGGGCCAGCCTAGAGAGCCTCAGGTCTGCACCC TGCCTCCCAGCCGGGAAGAGATGACCAAGAACCAGGTGT CCCTGAGCTGCGCCGTGAAGGGCTTCTACCCCTCCGATAT CGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACA ACTACAAGACCACCCCTCCCGTGCTGGACAGCGACGGCA GCTTCTTCCTGGTGTCCAAACTGACCGTGGACAAGAGCCG GTGGCAGCAGGGCAATGTGTTTCAGCTGTAGCGTGATGCA CGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTGAG CCTGAGCCCTGGCAA
SEQ ID NO: 26	α hCSF1R cabiralizumab VH	CAGGTGCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAAA CCTGGCTCCTCCGTGAAGGTGTCCTGCAAGGCTTCTGGCT ACACCTTTACCGACAACATACATGATCTGGGTCCGACAGG CTCCTGGACAGGGACTTGAGTGGATGGGCGACATCAACC CTTACAACGGCGGCACCACCTTCAACCAGAAATTCAAGG GCAGAGTGACCATCACCGCCGACAAGTCTACCTCCACCG CCTACATGGAAGTGTCCAGCCTGAGATCTGAGGACACCG CCGTGTACTACTGCGCCAGAGAGTCCCCTTACTTCTCCAA CCTGTACGTGATGGACTACTGGGGCCAGGGCACACTGGT CACAGTGTCTCT
SEQ ID NO: 27	α hCSF1R cabiralizumab VL	GAGATCGTGCTGACCCAGTCTCCTGCCACACTGTCACTGT CTCCAGGCGAGAGAGCTACCCTGTCCTGCAAGGCTTCTC AGTCCGTGGACTACGACGGCGACAACATGAAGTGGT ATCAGCAGAAGCCCGGCCAGGCTCCTAGACTGCTGATCT ACGCCGCCTCCAACCTGGAATCTGGCATCCCCGCTAGATT CTCCGGCTCTGGCTCTGGCACAGACTTTACCCTGACCATC TCCAGCCTGGAACCTGAGGACTTCGCCGTGTACTACTGCC ACCTGTCCAACGAGGACCTGTCCACATTTGGCGGAGGCA CCAAGGTGGAAATCAAG

Table 2. Sequences used to construct ORFs.

Full length	Variable	Constant	Fc
SEQ ID NO: 28	SEQ ID NO: 1	SEQ ID NO: 3	SEQ ID NO: 9
SEQ ID NO: 29	SEQ ID NO: 2	SEQ ID NO: 4	
SEQ ID NO: 30	SEQ ID NO: 5	SEQ ID NO: 6	SEQ ID NO: 10

SEQ ID NO: 31	SEQ ID NO: 7	SEQ ID NO: 8	
SEQ ID NO: 32	SEQ ID NO: 11	SEQ ID NO: 12	SEQ ID NO: 13
SEQ ID NO: 33	SEQ ID NO: 14	SEQ ID NO: 15	
SEQ ID NO: 34	SEQ ID NO: 16	SEQ ID NO: 12	SEQ ID NO: 13
SEQ ID NO: 35	SEQ ID NO: 17	SEQ ID NO: 18	
SEQ ID NO: 36	SEQ ID NO: 19	SEQ ID NO: 12	SEQ ID NO: 13
SEQ ID NO: 37	SEQ ID NO: 20	SEQ ID NO: 15	
SEQ ID NO: 38	SEQ ID NO: 21	SEQ ID NO: 12	SEQ ID NO: 13
SEQ ID NO: 39	SEQ ID NO: 22	SEQ ID NO: 18	
SEQ ID NO: 40	SEQ ID NO: 23	SEQ ID NO: 12	SEQ ID NO: 25
SEQ ID NO: 41	SEQ ID NO: 24	SEQ ID NO: 15	
SEQ ID NO: 42	SEQ ID NO: 26	SEQ ID NO: 12	SEQ ID NO: 25
SEQ ID NO: 43	SEQ ID NO: 27	SEQ ID NO: 15	

Table 3. Nucleic acid sequences of ORFs.

	Nucleic Acid Sequence
SEQ ID NO: 28	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGACAGGTCCAGCTGCAAGAGTCTGGCCCTGGACTGGTTCA GCCCTCTCAGACCCTGTCTCTGACCTGTACCGTGTCCGGCTTCTCCCTGAC CGACTTCTCTGTGCACTGGGTCCGACAGCCTCCAGGCAAAGGACTGGAA TGGATGGGCAGAATCAGATCCGAGGGCAACACCGACTACAACAGCGCCC TGAAGTCCCGGCTGTCTATCAGCAGAGACACCTCCAAGAGCCAGGTGTT CCTGAAGATGAACTCCCTGCAGACCGAGGACACCGCCATCTATTTCTGCA CCAGAGGCGACATCCTCGGCTTCGGCTATTGGGGACAGGGCGTGATGGT CACCGTTAGCTCTGCTCAGACCACCGCTCCTAGCGTGTACCCTTTGGCTC CTGGCTGTGGCGACACCACCTCTTCTACAGTGACCCTGGGCTGTCTGGTC AAGGGCTACTTTCCTGAGCCTGTGACCGTGACCTGGAACCTCTGGTGCCCT GTCCTCCGACGTGCACACCTTTCCAGCTGTGCTGCAGTCCGGCCTGTACA CCCTGACATCCTCCGTGACCTCTTCCACCTGGCCTAGCCAGACCGTGACA TGCAATGTGGCTCACCTGCCTCCAGCACCAAGGTGGACAAGAAGGTGG AACGGCGGACCATTAAGCCTTGTCTCCATGCAAGTGCCCCGCTCCTAAT CTGCTCGGAGGCCCTTCCGTGTTTCATCTTTCCACCTAAGATCAAGGACGT GCTGATGATCTCCCTGTCTCCTATCGTGACCTGCGTGGTGGTGGACGTGT CCGAGGATGATCCTGACGTGCAGATCAGTTGGTTCGTGAACAACGTGGA AGTGCACACCGCTCAGACCCAGACACACAGAGAGGACTACAACCTCTACC CTGAGAGTGGTGTCTGCCCTGCCTATCCAGCATCAGGACTGGATGTCCGG CAAAGAATTCAAGTGCAAAGTGAACAACAAGGACCTGCCTGCTCCAATC GAGCGGACCATCTCTAAGCCTAAGGGCTCTGTCAGGGCCCCCTCAGGTGT ACGTTCTGCCTCCTTGCGAGGAAGAGATGACCAAGAAACAAGTGACACT GTGGTGCATGGTCACAGACTTCATGCCCGAGGACATCTACGTGGAATGG ACCAACAACGGCAAGACCGAGCTGAACTACAAGAACACCGAGCCTGTGC TGGACTCCGACGGCTCCTACTTCATGTACTCCAAGCTGCGCGTCGAGAAG AAGAAGTGGGTGCGAGAGAACTCCTACTCCTGCTCCGTGGTGCACGAGG GCCTGCACAATCACACACCAAGTCCTTCTCTCGGACCCCTGGCAAG TGATGA
SEQ ID NO: 29	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACCGGCGACATCGTGATGACCCAGTCTCCACTGTCCGTGTCTGTG

	ACCCCTGGCGAGTCTGCCTCCATCTCCTGCAGATCCTCCAAGAGCCTGCT GCACTTCAAGGGGCATCACCTTCGTGTACTGGTATCTGCAGAAGCCCCGGCC AGTCTCCTCAGCTGCTGATCTTCAGAATGTCCAGCCTGGCCTCTGGCGTG CCCGATAGATTTTCTGGCTCCGGCTCCGAGACAGACTTCACCCTGAAGAT CTCCAGAGTGGAAGCCGAGGACGTGGGCACCTACTATTGTGGCCAGCTG CTGGAAAACCCCTACACCTTTGGCGCTGGCACCAAGCTGGAACTGAAGA GAGCTGACGCTGCCCCCTACCGTGTCTATCTTCCCTCCATCCATGGAACAG CTGACCTCTGGCGGAGCTACCGTCGTGTGCTTCGTGAACAACCTTCTACCC TCGGGACATCTCCGTGAAGTGGAAGATCGACGGCTCTGAGCAGCGAGAT GGCGTGCTGGATTCTGTGACCGACCAGGACTCCAAGGACAGCACCTACT CCATGTCTAGCACCTGAGCCTGACCAAGGTGGAATACGAGCGGCACAA CCTGTATACCTGCGAGGTGGTGCACAAGACCTCCAGCTCTCCCGTGGTCA AGTCCTTCAACCGGAACGAGTGCTGATGA
SEQ ID NO: 30	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGACAGGTCCAGTTGCAGCAGTCTGGCGCTGAGCTGGTCAA GCCTGGATCCTCCGTGAAGATCTCCTGCAAGGCCTCCGGCTACACCTTCA CCTCCAACCTTCATGCACTGGATCAAGCAGCAGCCCCGGCAACGGCCTGGA ATGGATCGGATGGATCTATCCTGGCGACGGCGACACCGAGTACAACCAG AAGTTCAACGGCAAGGCTACCCTGACCGCCGACAAGTCCTCTTCCACCG CTTACATGCAGCTGTCCAGCCTGACCTCTGAGGACTCCGCCGTGTACTTC TGCGCCGTGAATTATGGCGGCTACGTGCTGGATGCTTGGGGCCAAGGCG CTTCTGTGACAGTGTCTCTGCCGAGACAACCGCTCCTAGCGTTTACCCT CTGGCTCCTGGCACAGCCCTGAAGTCCAACCTCTATGGTCACCCTGGGCTG CCTGGTCAAGGGCTACTTTCCTGAGCCTGTGACCGTGACCTGGAACCTCTG GTGCTCTGTCTAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAGAGCGGC CTGTACACCCTGACATCTAGCGTGACCGTGCCCTTCCAGCACCTGGTCTAG TCAGGCTGTGACCTGCAACGTGGCCCATCCTGCCTCTTCTACCAAGGTGG ACAAGAAAATCGTGCCCAGAGAGTGCAACACCATCAAGCCCTGTCCTCC ATGCAAGTGCCCCGCTCCTAATCTGCTCGGAGGCCCTTCCGTGTTTCATCT TCCCACCTAAGATCAAGGACGTGCTGATGATCTCCCTGTCTCCTATCGTG ACCTGCGTGGGTGGTGGACGTGTCCGAGGATGATCCTGACGTGCAGATCA GTTGGTTCGTGAACAACGTGGAAGTGCACACCGCTCAGACCCAGACACA CAGAGAGGACTACAACAGCACCTGAGAGTGGTGTCTGCCCTGCCAATC CAGCACCAGGATTGGATGTCCGGCAAAGAATTCAAGTGCAAAGTGAACA ACAAGGACCTGCCTGCTCCAATCGAGCGGACCATCTCTAAGCCTAAGGG CTCTGTGCGGGCTCCCCAAGTTTGTGTTCTGCCTCCACCTGAGGAAGAGA TGACCAAGAAACAAGTGACCCTGTCTTGTGCCGTGACCGACTTCATGCCC GAGGACATCTACGTGGAATGGACCAACAATGGCAAGACCGAGCTGAACT ACAAGAACACCGAGCCTGTGCTGGACTCCGACGGCTCCTACTTCATGGT GTCTAAGCTGCGCGTCGAGAAGAAGAAGTGGGTCGAGAGAACTCCTAC TCCTGCTCCGTGGTGCACGAGGGCCTGCACAATCACCACACCACCAAGT CCTTCTCTCGGACCCCTGGCAAGTGATGA
SEQ ID NO: 31	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGCGAGATCGTGCTGACCCAGTCTCCTACCACCATGGCTGCT AGCCCTGGCGAGAAAGTGACAATTACCTGCCGGGCCTCCTCCTCCACCA ACTACATGTCCTGGTATCAGCAGAAGTCCGGCGCCTCTCCTAAGCCTTGG ATCTACGAGACATCCAAGCTGGCCTCTGGCGTGCCCGATAGATTTTCCGG CTCTGGCTCCGGCACCTCCTACAGCTTCACCATCTCCAGCATGGAAACAG AGGACGCCGCCACCTACTACTGCCACCAGTGGTCATCTACCCCTCTGACC TTTGGCAGCGGCACCAAGCTGGAAATCAAGAGAGCTGACGCCGCTCCTA CCGTGTCTATCTTCCCTCCATCCATGGAACAGCTGACCTCCGGCGGAGCT

	ACCGTCGTGTGTTTCGTGAACAACCTTCTACCCTCGGGACATCTCCGTGAA GTGGAAGATCGACGGCTCTGAGCAGCGAGATGGCGTGCTGGATTCTGTG ACCGACCAGGACTCCAAGGACAGCACCTACTCCATGTCTAGCACCTGA GCCTGACCAAGGTGGAATACGAGCGGCACAACCTGTATACCTGCGAGGT GGTGCAACAAGACCTCCAGCTCTCCCGTGGTCAAGTCCTTCAACCGGAAC GAGTGCTGATGA
SEQ ID NO: 32	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGCGAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTAA GCCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCTTCTGGCTTCACCTTCTC CGCCTACGCCATGAACTGGGTCCGACAGGCTCCTGGCAAAGGCCTGGAA TGGGTCGGAAGAATCCGGACCAAGAACAACAACCTACGCCACCTACTACG CCGACTCCGTGAAGGACCGGTTACCATCTCTCGGGACGACTCCAAGAA CACCTGTACCTGCAGATGAACTCCCTGAAAACCGAGGACACCGCCGTG TACTACTGCACCACCTTCTACGGCAATGGCGTGTGGGGACAGGGGCACAC TGGTTACCGTTTCTTCCGCCTCCACCAAGGGACCCTCTGTGTTTCCTCTGG CTCCCTCCAGCAAGTCTACCTCTGGTGGAACAGCTGCCCTGGGCTGCCTG GTCAAGGATTACTTTCCTGAGCCTGTGACCGTGTCCCTGGAACCTCTGGCGC TCTGACATCTGGCGTGCACACCTTTCAGCTGTGCTGCAGTCCTCTGGCC TGTA CTCTCTGTCCTCCGTCGTGACCGTGCCTTCTAGCTCTCTGGGGACCC AGACCTACATCTGCAATGTGAACCACAAGCCTTCCAACACCAAGGTGGA CAAGAGAGTGGAACCCAAGTCCTGCGACAAGACCCACACCTGTCCTCCA TGTCCTGCTCCAGAACTGCTCGGCGGACCTTCCGTGTTCCCTGTTTCCTCCA AAGCCTAAGGACACCCTGATGATCTCTCGGACCCCTGAAGTGACCTGCG TGGTGGTGGATGTGTCTCACGAGGATCCCGAAGTGAAGTTCAATTGGTA CGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGA ACAGTACAACCTCCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACC AGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGG CCCTGCCTGCTCCTATCGAAAAGACCATCTCCAAGGCCAAGGGCCAGCC TAGGGAACCCAGGTTTACACCCTGCCTCCATGCCGGGAAGAGATGACC AAGAATCAGGTGTCCCTGTGGTGCCTCGTGAAGGGCTTCTACCCTTCCGA TATCGCCGTGGAATGGGAGAGCAATGGCCAGCCTGAGAACAACCTACAAG ACAACCCCTCCTGTGCTGGACTCCGACGGCTCATTCTTCCTGTACTCCAA GCTGACAGTGGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCTCCTGC TCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGTCCCTGTC TCTGTCCCCTGGCAAGTGATGA
SEQ ID NO: 33	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGCGACGTGGTCATGACACAGAGCCCTCTGTCTCTGCCCCGTG ACATTGGGACAGCCTGCCTCCATCTCCTGCAAGTCCTCTCAGTCCCTGCT GGACTCTGACGGCAAGACCTTCCTGAACTGGTTCAGCAGCGGCCTGGC CAGTCTCCTAGAAGGCTGATCTACCTGGTGTCCAAGCTGGATTCTGGCGT GCCCCGACAGATTCTCCGGCTCTGGCTCTGGCACCGACTTCACCCTGAAGA TCTCCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTTGGCAGGG CACCCACTTTCATACACCTTCGGCCAGGGCACCAGACTGGAAATCAAG AGAACCGTGGCCGCTCCTTCCGTGTTTCATCTTCCCACCTTCCGACGAGCA GCTGAAGTCCGGCACAGCTTCTGTCTGTGCCTGCTGAACAACCTTCTACC CTCGGGAAGCCAAGGTGCAGTGGAAAGGTGGACAATGCCCTGCAGTCCGG CAACTCCCAAGAGTCTGTGACCGAGCAGGACTCCAAGGACAGCACCTAC AGCCTGTCCAGCACACTGACCCTGTCCAAGGCCGACTACGAGAAGCACA AGGTGTACGCCTGCGAAGTGACCCATCAGGGCCTGTCTAGCCCTGTGAC CAAGTCTTTCAACCGGGGCGAGTGCTGATGA
SEQ ID NO: 34	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG

	GATCTACAGGCGAAGTGCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAA ACCTGGCGCCTCTGTGAAGGTGTCCTGCAAGGCTTCTGGCTACACCTTTA CCGGCTACCACATGCACTGGGTCCGACAGGCTCCAGGACAAGGCTTGGA ATGGATGGGCTGGATCAACCCCAACTCCGGCGTGACCAAATACGCCCAG AAATTCCAGGGCAGAGTGACCATGACCAGAGACACCTCCATCAACACCG CCTACATGGAAGTGTCCCGGCTGAGATTGACGACACCGACGTGTACTA CTGTGCCACCGGCGGCTTTGGCTATTGGGGAGAGGGAACACTGGTCACC GTGTCCTCCGCTTCTACCAAGGGACCCTCCGTGTTTCCTCTGGCTCCTTCC AGCAAGTCTACCTCCGGTGGAACAGCTGCTCTGGGCTGCCTGGTCAAGG ACTACTTTCCTGAGCCTGTGACCGTGTCTTGGAAGTCTGGCGCTCTGACA TCCGGCGTGCACACCTTTCCAGCTGTGCTGCAATCCTCCGGCCTGTACTC TCTGTCCTCCGTCGTGACCGTGCCTTCTAGCTCTCTGGGCACCCAGACCT ACATCTGCAATGTGAACCACAAGCCTTCCAACACCAAGGTGGACAAGAG AGTGGAACCCAAGTCCTGCGACAAGACCCACACCTGTCCTCCATGTCCTG CTCCAGAACTGCTCGGCGGACCTTCTGTGTTTCCTGTTTCCTCCAAAGCCT AAGGACACCCTGATGATCTCTCGGACCCCTGAAGTGACCTGCGTGGTGG TGGATGTGTCTCACGAGGACCCAGAAGTGAAGTTCAATTGGTACGTGGA CGGCGTGGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGTA CAACTCCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATT GGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCC TGCTCCTATCGAAAAGACCATCTCCAAGGCCAAGGGCCAGCCTAGGGAA CCCCAGGTTTACACCCTGCCTCCATGCCGGGAAGAGATGACCAAGAACC AGGTGTCCCTGTGGTGCCTCGTGAAGGGCTTCTACCCTTCCGATATCGCC GTGGAATGGGAGAGCAATGGCCAGCCTGAGAACAACACTACAAGACAACC CCTCCTGTGCTGGACTCCGACGGCTCATTCTTCCTGTACTCCAAGCTGAC AGTGGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG ATGCACGAGGCCCTGCACAATCACTACACACAGAAGTCCCTGTCTCTGTC CCCTGGCAAGTGATGA
SEQ ID NO: 35	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGACTGCCCCGTGTTGACCCAGCCTCCTAGCGTTTCCAAGGGC CTGAGACAGACCGCCACACTGACCTGTACCGGCAACTCTAACAACGTGG GCAATCAGGGCGCTGCCTGGTTGCAGCAGCATCAGGGACAGCCTCCAAA GCTGCTGTCCTACCGGAACCAACAACAGACCTAGCGGGCGTGTCCGAGCGG TTCAGCCCTTCTAGATCTGGCGACACCTCCAGCCTGACCATCACTGGACT GCAGCCTGAGGACGAGGCCGACTACTATTGTCTGGCCTGGGACAGCTCC CTGCGGGCCTTTGTTTTTGGCACCGGCACCAAGCTGACCGTGCTGGGACA ACCTAAGGCCAATCCTACCGTGACACTGTTCCCTCCATCCTCCGAGGAAC TGCAGGCCAACAAGGCTACCCTCGTGTGCCTGATCTCCGACTTTTACCCT GGCGCTGTGACCGTGGCCTGGAAGGCTGATGGATCTCCTGTGAAGGCTG GCGTGGAACCAACCAAGCCTTCCAAGCAGTCCAACAACAAATACGCCGC CTCCTCCTACCTGTCTCTGACCCCTGAACAGTGGAAGTCCCACCGGTCTC ACAGCTGCCAAGTGACCCATGAGGGCTCCACCGTGGAAGAACCGTGGC TCCTACCGAGTGCTCCTGATGA
SEQ ID NO: 36	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGACAGGTGCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAA ACCTGGCGCCTCTGTGAAGGTGTCCTGCAAGGCTTCCGGCTACACCTTCT CCAGCTACTACATGCACTGGGTCCGACAGGCCCTGGACAAGGATTGGA GTGGATGGGCATCATCAACCCCTCTGGCGGCAACACCTCTTACGCCCAG AAATTCCAGGGCAGAGTGACCATGACCAGAGACACCTCCACCAGCACCG TGTACATGGAAGTGTCCAGCCTGAGATCCGAGGACACCGCCGTGTACTA CTGTGCCAGAGGCGGATACCAGCTGCCTCACGGTAGAGCCAGAGCCTTC

	GATATGTGGGGCCAGGGCACAATGGTCACCGTGTCTCTGCTTCCACCAA GGGACCCTCTGTGTTCCCTCTGGCTCCTTCCAGCAAGTCCACATCCGGTG GAACAGCTGCTCTGGGCTGCCTGGTCAAGGACTACTTTCCTGAGCCTGTG ACCGTGTCTTGGAACCTCTGGCGCTCTGACATCCGGCGTGCACACATTTCC AGCTGTGCTGCAGTCCTCCGGCCTGTACTCTCTGTCTCTGTCTGACCGT GCCTTCCAGCTCTCTGGGAACCCAGACCTACATCTGCAATGTGAACCACA AGCCTTCCAACACCAAGGTGGACAAGAGAGTGGAAACCCAAGTCCTGCGA CAAGACCCACACCTGTCCACCATGTCTGCTCCAGAACTGCTCGGCGGAC CTTCCGTGTTCCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATCTCTC GGACCCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCCCACGAGGACCC AGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCC AAGACCAAGCCTAGAGAGGAACAGTACAACCTCCACCTACAGAGTGGTGT CCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAAGAGTACAA GTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCCTATCGAAAAGACCATC TCCAAGGCCAAGGGCCAGCCTAGGGAACCCCAGGTTTACACCCTGCCTC CATGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGTGGTGCCTCGT GAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGGGAGAGCAATGGC CAGCCAGAGAACAACTACAAGACAACCCCTCCTGTGCTGGACTCCGACG GCTCATTCTTCCTGTACTCCAAGCTGACAGTGGACAAGTCCAGATGGCAG CAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAATCA CTACACACAGAAGTCCCTGTCTCTGTCCCCTGGCAAGTGATGA
SEQ ID NO: 37	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACCGGCGCCATCAGAATGACCCAGTCTCCACTGAGCCTGCCTGTG ACATTGGGCCAGCCTGCCTCTATCTCCTGCACCTCCTCTCAGTCTCTGGTG TACAGAGATGGCACCACCTACCTGAACTGGTTCAGCAGAGGCCTGGCC AGTCTCCTAGACGGCTGATCTACAAGGTGTCCAACAGAGACTCTGGCGT GCCCCGACAGATTCACCGGCTCTGGCTCTGGCACCACATTCACCCTGACCA TCTCCAGAGTGGAAGCCGAGGACGTGGGCATCTACTACTGTATGCAGGG CACCCACTGGCCTCTGACCTTTGGCCAGGGAACAAAGGTGGAAATCAAG CGGACCGTGGCCGCTCCTTCCGTGTTTCATCTTCCCACCTTCCGACGAGCA GCTGAAGTCTGGCACAGCCTCTGTCTGTGCTGCTGAACAACCTTCTACC CTCGGGAAGCCAAGGTGCAGTGGAAAGGTGGACAATGCCCTGCAGTCCGG CAACTCCCAAGAGTCTGTGACCGAGCAGGACTCCAAGGACAGCACCTAC AGCCTGTCCTCCACACTGACCCTGTCCAAGGCCGACTACGAGAAGCACA AGGTGTACGCCTGCGAAGTGACCCATCAGGGCCTGTCTAGCCCTGTGAC CAAGTCTTTCAACCGGGGGCGAGTGCTGATGA
SEQ ID NO: 38	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGCGAGGTGCAGCTGGTTGAATCTGGCGGAGGATTGGTTCA GCCTGGCGGCTCTCTGAGACTGTCTTGTGTGGCCTCTGGCTTCACCTTCTC CGACTACTGGATGTCCTGGGTCCGACAGGCTCCTGGCAAAGGACTGGAA TGGGTCGCCAACATCAAGAAAGACGGCTCCGTGAACTACTACGTGGACT CCGTGAAGGGCAGATTCACCATCTCTCGGGACAACGCCAAGAAGTCCCT GTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTAC TGCACCAGATTCGATTACTGGGGCCAGGGCACCCCTGGTCACAGTGTCTC TGCTTCTACCAAGGGACCCAGCGTGTTCCCTCTGGCTCCTTCCAGCAAGT CTACCTCTGGCGGAACAGCTGCTCTGGGCTGCCTGGTCAAGGACTACTTT CCTGAGCCTGTGACCGTGTCTGGAACCTCTGGCGCTCTGACATCTGGCGT GCACACCTTTCCAGCTGTGCTGCAGTCCTCCGGCCTGTACTCTCTGTCTC TGTCGTGACCGTGCCTTCCAGCTCTCTGGGAACCCAGACCTACATCTGCA ATGTGAACCACAAGCCTTCCAACACCAAGGTGGACAAGAGAGTGGAAACC CAAGTCCTGCGACAAGACCCACACCTGTCCTCCATGTCTGCTCCAGAAC

	TGCTCGGCGGACCTTCCGTGTTCCCTGTTTCCTCCAAAGCCTAAGGACACC CTGATGATCTCTCGGACCCCTGAAGTGACCTGCGTGGTGGTGGATGTGTC TCACGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGA GTGCACAATGCCAAGACCAAGCCTAGAGAGGAACAGTACAACCTCCACCT ACAGAGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGG CAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCCTATC GAAAAGACCATCTCCAAGGCCAAGGGCCAGCCTAGGGAACCCCAAGGTTT ACACCCTGCCTCCATGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCT GTGGTGCCTGGTTAAGGGCTTCTACCCCTCCGATATCGCCGTGGAATGGG AGTCTAATGGCCAGCCAGAGAACAACACTACAAGACAACCCCTCCTGTGCT GGA CTCCGACGGCTCATTCTTCCTGTACTCCAAGCTGACAGTGGACAAGT CCAGATGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGC CCTGCACAATCACTACACCCAGAAGTCCCTGTCTCTGTCCCCTGGCAAGT GATGA
SEQ ID NO: 39	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGACAGGCTGGCTTGACCCAGCCTCCTAGCGTTTCCAAGGG CCTGAGACAGACCGCCACACTGACCTGTACCGGCAACTCTAACAACGTG GGCAATCAGGGCGCTGCCTGGTTGCAGCAGCATCAGGGACATCCTCCAA AGCTGCTGTTCTACCGGAACAACAACAGAGCCTCCGGCATCTCCGAGCG GCTGTCTGCTTCTAGATCCGGCAATACCGCCAGCCTGACCATCACTGGAC TGCAGCCTGAGGACGAGGCCGACTACTATTGCCTGACCTGGGACTCCTCT CTGTCCGTGGTGGTTTTTGGCGGAGGCACCAAGCTGACAGTGCTGGGAC AGCCTAAGGCCAATCCTACCGTGACACTGTTCCCTCCATCCTCCGAGGAA CTGCAGGCCAACAAGGCTACCCTCGTGTGCCTGATCTCCGACTTTTACCC TGGCGCTGTGACCGTGGCCTGGAAGGCTGATGGATCTCCTGTGAAGGCT GGCGTGGA AACCACCAAGCCTTCCAAGCAGTCCAACAACA AATACGCCG CCTCCTCCTACCTGTCTCTGACCCCTGAACAGTGGAAGTCCCACCGGTCC TACAGCTGCCAAGTGACCCATGAGGGCTCCACCGTGGA AAGACCGTGG CTCCTACCGAGTGCTCCTGATGA
SEQ ID NO: 40	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGACAGGTGCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAA ACCTGGCGCCTCTGTGAAGGTGTCCTGCAAGGCTTCCGGCTACACCTTTA CCAGCTACGACATCTCCTGGGTCCGACAGGCTCCTGGACAAGGCTTGGA ATGGATGGGCGTGATCTGGACCGATGGCGGCACCAATTACGCCCAGAAA CTGCAGGGCAGAGTGACCATGACCACCGACACCTCTACCTCCACCGCCT ACATGGA ACTGCGGTCCCTGAGATCTGACGACACCGCCGTGTACTACTG CGCCAGAGATCAGCGGCTGTACTTCGATGTGTGGGGCCAGGGCACAACC GTGACAGTGTCCCTCTGCTTCCACCAAGGGACCCAGCGTTTTCCCTCTGGC TCCATCCTCCAAGTCTACCTCTGGCGGAACAGCTGCTCTGGGCTGCCTGG TCAAGGACTACTTTCCCTGAGCCTGTGACCGTGTCCCTGGA ACTCTGGCGCT CTGACATCTGGCGTGCACACATTCCCTGCTGTGCTGCAGTCCTCCGGCCT GTACTCTCTGTCCTCTGTGGTTACCGTGCCTTCCTCTAGCCTGGGCACCCA GACCTACATCTGCAATGTGAACCACAAGCCTTCCAACACCAAGGTGGAC AAGAGAGTGGAACCCAAGTCCTGCGACAAGACCCACACCTGTCCACCAT GTCCTGCTCCAGAACTGCTCGGCGGACCTTCCGTGTTCCCTGTTTCCTCCA AAGCCTAAGGACACCCTGATGATCTCTCGGACCCCTGAAGTGACCTGCG TGGTGGTGGATGTGTCTCACGAGGACCCAGAAGTGAAGTTCAATTGGTA CGTGGACGGCGTGGAAGTGCAACGCCAAGACCAAGCCTAGAGAGGA ACAGTACA ACTCCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACC AGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGG CCCTGCCTGCTCCTATCGAAAAGACCATCTCCAAGGCCAAGGGCCAGCC

	TCGGGAACCTCAAGTCTGTACCCTGCCTCCTAGCCGGGAAGAGATGACC AAGAACCAGGTGTCCCTGAGCTGCGCCGTGAAGGGCTTCTACCCTTCTGA TATCGCCGTGGAATGGGAGAGCAACGGCCAGCCTGAGAACAACACTACAAG ACAACCCCTCCTGTGCTGGACTCCGACGGCTCATTCTTCCTGGTGTCCAA GCTGACAGTGGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCTCCTGC TCCGTGATGCACGAGGCCCTGCACAATCACTACACACAGAAGTCCCTGT CTCTGTCCCCTGGCAAGTGATGA
SEQ ID NO: 41	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACCGGCGACATCCAGATGACCCAGTCTCCATCCTCTCTGTCCGCC TCTGTGGGCGACAGAGTGACCATCACCTGTAGAGCCTCCGAGGACGTGA ACACCTACGTGTCCTGGTATCAGCAGAAGCCCCGGCAAGGCTCCCAAGCT GCTGATCTACGCCGCCTCTAACAGATAACACCGGCGTGCCCTCTAGATTCT CCGGCTCTGGCTCTGGCACCGACTTTACCCTGACAATCTCCAGCCTGCAG CCTGAGGACTTCGCCACCTACTACTGCCAGCAGTCCTTCAGCTACCCAC CTTTGGCCAGGGCACCAAGCTGGAAATCAAGCGGACAGTGGCCGCTCCT TCCGTGTTTCATCTTCCCACCTTCCGACGAGCAGCTGAAGTCCGGCACAGC TTCTGTCGTGTGCCTGCTGAACAACCTTCTACCCTCGGGAAGCCAAGGTGC AGTGGAAGGTGGACAATGCCCTGCAGTCCGGCAACTCCCAAGAGTCTGT GACCGAGCAGGACTCCAAGGACAGCACCTACAGCCTGTCCTCCACACTG ACCCTGTCCAAGGCCGACTACGAGAAGCACAAAGGTGTACGCCTGCGAAG TGACCCATCAGGGCCTGTCTAGCCCTGTGACCAAGTCTTTCAACCGGGGC GAGTGCTGATGA
SEQ ID NO: 42	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGACAGGTGCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAA ACCTGGCTCCTCCGTGAAGGTGTCCTGCAAGGCTTCTGGCTACACCTTTA CCGACAACCTACATGATCTGGGTCCGACAGGCTCCTGGACAGGGACTTGA GTGGATGGGCGACATCAACCCTTACAACGGCGGCACCACCTTCAACCAG AAATTCAAGGGCAGAGTGACCATCACCGCCGACAAGTCTACCTCCACCG CCTACATGGAACTGTCCAGCCTGAGATCTGAGGACACCGCCGTGTACTA CTGCGCCAGAGAGTCCCCTTACTTCTCCAACCTGTACGTGATGGACTACT GGGGCCAGGGCACACTGGTCACAGTGTCTCTGCTTCCACCAAGGGACC CAGCGTTTTTCCCTCTGGCTCCATCCTCCAAGTCCACCTCTGGTGGAACAG CTGCTCTGGGCTGCCTGGTCAAGGACTACTTTCCTGAGCCTGTGACCGTG TCCTGGAACCTCTGGCGCTCTGACATCTGGCGTGCACACCTTTCCAGCTGT GCTGCAGTCCTCCGGCCTGTACTCTCTGTCTCTGTCGTGACCGTGCCCTC CAGCTCTCTGGGAACCCAGACCTACATCTGCAATGTGAACCACAAGCCTT CCAACACCAAGGTCGACAAGAGAGTGGAACCCAAGTCCTGCGACAAGA CCCACACCTGTCCACCTTGTCTCTGCTCCAGAACTGCTCGGCGGACCTTCC GTGTTCTCTGTTTCCCTCCAAAGCCTAAGGACACCCTGATGATCTCTCGGAC CCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCTCACGAGGACCCAGAA GTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGA CCAAGCCTAGAGAGGAACAGTACAACCTCCACCTACAGAGTGGTGTCCGT GCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAAGAGTACAAGTGC AAGGTGTCCAACAAGGCCCTGCCTGCTCCTATCGAAAAGACCATCTCCA AGGCCAAGGGCCAGCCTCGGGAACCTCAAGTCTGTACCCTGCCTCCTAG CCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGAGCTGCGCCGTGAAG GGCTTCTACCCTTCTGATATCGCCGTGGAATGGGAGAGCAACGGCCAGC CAGAGAACAACACTACAAGACAACCCCTCCTGTGCTGGACTCCGACGGCTC ATTCTTCCTGGTGTCCAAGCTGACAGTGGACAAGTCCAGATGGCAGCAG GGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTA CACACAGAAGTCTCTGTCTCTGAGCCCCGGCAAGTGATGA

SEQ ID NO: 43	ATGGAAACCGACACACTGCTGCTGTGGGTGCTGCTCTTGTGGGTGCCAG GATCTACAGGCGAGATCGTGCTGACCCAGTCTCCTGCCACACTGTCACTG TCTCCAGGCGAGAGAGCTACCCCTGTCCTGCAAGGCTTCTCAGTCCGTGGA CTACGACGGCGACAACACTACATGAACTGGTATCAGCAGAAGCCCGGCCAG GCTCCTAGACTGCTGATCTACGCCGCCTCCAACCTGGAATCTGGCATCCC CGCTAGATTCTCCGGCTCTGGCTCTGGCACAGACTTTACCCTGACCATCT CCAGCCTGGAACCTGAGGACTTCGCCGTGTACTACTGCCACCTGTCCAAC GAGGACCTGTCCACATTTGGCGGAGGCACCAAGGTGGAAATCAAGCGGA CAGTGGCCGCTCCTTCCGTGTTTCATCTTCCCACCTTCCGACGAGCAGCTG AAGTCTGGCACCGCTTCTGTTCGTGTGCCTGCTGAACAACCTTCTACCCTCG GGAAGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGTCCGGCAAC TCCAAGAGTCTGTGACCGAGCAGGACTCCAAGGACAGCACCTACAGCC TGTCCTCCACACTGACCCTGTCCAAGGCCGACTACGAGAAGCACAAGGT GTACGCCTGCGAAGTGACCCATCAGGGCCTGTCTAGCCCTGTGACCAAG TCTTTCAACCGGGGCGAGTGCTGATGA
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2. Expression and Purification.

The plasmids were co-transfected into either Expi293 cells (Life Technologies A14527) or ExpiCHO cells (Life Technologies A29127). Transfections were performed using 1 mg of total DNA for a multispecific construct with a 1:1 knob to hole heavy chain ratio and 3:2 light chain to heavy chain ratio. When biotinylation was required, 250 µg of BirA was added per liter in addition to the multispecific construct DNA. Transfection in Expi293 cells was done using linear 25,000 Da polyethylenimine (PEI, Polysciences Inc 23966) in a 3:1 ratio with the total DNA. The DNA and PEI were each added to 50 mL of OptiMem (Life Technologies 31985088) medium and sterile filtered. The DNA and PEI were combined for 10 minutes and added to the Expi293 cells with a cell density of 1.8 – 2.8 x 10⁶ cells/mL and a viability of at least 95 %. The ExpiCHO transfection was performed according to the manufacturer’s instructions. Expi293 cells were grown in a humidified incubator at 37 °C with 8 % CO₂ for 5-7 days after transfection and ExpiCHO cells were grown for 14 days at 32 °C with 5 % CO₂. The cells were pelleted by centrifugation at 4500 x g and the supernatant was filtered through a 0.2 µm membrane. Protein A resin (GE 17-1279-03) was added to the filtered supernatant and incubated for 1-3 hours at room temperature. The resin was packed into a column, washed with 3 x 10 column volumes of Dulbecco’s phosphate-buffered saline (DPBS, Life Technologies 14190-144). The bound protein was eluted from the column with 20 mM citrate, 100 mM NaCl, pH 2.9. When necessary, the proteins were further purified using ligand affinity and/or size exclusion chromatography on a Superdex 200 column with a running buffer of DPBS.

Table 4. Amino Acid Sequences.

SEQ ID NO	Description	Amino Acid Sequence
SEQ ID NO: 44	α CCR2 MC12 VH	QVQLQESGPGLVQPSQTLSTCTVSGFSLTDFSVHWVRQPP GKGGLEWMGRIRSEGNTDYN SALKSRLSISRDTSKSQVFLKM NSLQTEDTAIYFCTRGDILGFGYWGQGVMVTVSS
SEQ ID NO: 45	α CCR2 MC12 VL	DIVMTQSPLSVSVTPGESASISCRSSKSLHFKGITFVYWYLO KPGQSPQLLIFRMSSSLASGVPDRFSGSGSETDFTLKISRVEAE DVGTYYCGQLLENPYTFGAGTKLELK
SEQ ID NO: 46	R2b CH1	AQTTAPSVYPLAPGCGDTTSSTVTLGCLVKGYFPEPVTVTW NSGALSSDVHTFPAVLQSGLYTLTSSVTSSTWPSQTVTCNV AHPASSTKVDDKKVERR
SEQ ID NO: 47	R2b CL	RADAAPTVSIFPPSMEQLTSGGATVVCVNNFYPRDISVKW KIDGSEQRDGVLDSDVDQDSKDYSTYSMSSTLSLTKVEYERH NLYTCEVVHKTSSSPVVKSFNRNEC
SEQ ID NO: 48	α mCSF1R VH	QVQLQQSGAELVKPGSSVKISCKASGYTFTSNFMHWIKQQP GNGLEWIGWIYPGDGDEYNQKFNGKATLTADKSSSTAYM QLSSLTSEDSAVYFCAVNYGGYVLDAGWGQGASVTVSS
SEQ ID NO: 49	R2a CH1	AETTAPSVYPLAPGTALKSNMVTLGCLVKGYFPEPVTVTW NSGALSSGVHTFPAVLQSGLYTLTSSVTVPSSTWSSQAVTC NVAHPASSTKVDDKKIVPRECN
SEQ ID NO: 50	α mCSF1R VL	EIVLTQSPTTMAASPGEKVTITCRASSSTNYMSWYQQKSGA SPKPWIYETSKLASGVPDRFSGSGSGTSYSFTISSMETEDAAT YYCHQWSSTPLTFGSGTKLEIK
SEQ ID NO: 51	R2a CL	RADAAPTVSIFPPSMEQLTSGGATVVCVNNFYPRDISVKW KIDGSEQRDGVLDSDVDQDSKDYSTYSMSSTLSLTKVEYERH NLYTCEVVHKTSSSPVVKSFNRNEC
SEQ ID NO: 52	mFc Knob	TIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVV VDVSEDDPDVQISWVNNVEVHTAQTQTHREDYNSTLRVV SALPIQHQQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVR APQVYVLPPEEEMTKKQVTLWCMVTDFMPEDIYVEWTN NGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSY SCSVVHEGLHNHHTTKSFSRTPGK
SEQ ID NO: 53	mFc Hole	TIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVV VDVSEDDPDVQISWVNNVEVHTAQTQTHREDYNSTLRVV SALPIQHQQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVR APQVCVLPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTNN GKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYS CSVVHEGLHNHHTTKSFSRTPGK
SEQ ID NO: 54	α hCCR2 p10zalizumab VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMNWVRQA PGKGGLEWVGRIRTKNNNYATYYADSVKDRFTISRDDSKNTL YLQMNSLKTEDTAVYYCTTFYGNQVWGQGTLVTVSS
SEQ ID NO: 55	hCH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEPKSC
SEQ ID NO: 56	hFc Knob	DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVELTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE

		SNGQPENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 57	α hCCR2 plozalizumab VL	DVVMTQSPLSLPVTLGQPASISCKSSQSLLDSDGKTFLNWFQ QRPGQSPRRLIYLVSKLDSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCWQGT HFPYTFGQGTRLEIK
SEQ ID NO: 58	hCL (kappa)	RTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 59	α hCCR2 D1 VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTGYHMHWRQ APGQGLEWMGWINPNSGVTKYAQKFQGRVTMTDRDTSINTA YMELSR LRFDDTDVYYCATGGFGYWGEGLVTVSS
SEQ ID NO: 60	α hCCR2 D1 VL	LPVLTQPPSVSKGLRQTATLTCTGNSNNVGNQGAAWLQQH QGQPPKLLSYRNHNRP SGVSERFSPSRSGDTSSTLTITGLQPED EADYYCLAWDSSLRAFVFGTGTKLTVL
SEQ ID NO: 61	hCL (lambda)	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVA WKADGSPVKAGVETTKPSKQSN NKYAASSYLSLTPEQWKS HRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO: 62	α hCCR2 42G7 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFSSYYMHWRQ APGQGLEWMGIINPSGGNTSYAQKFQGRVTMTDRDTSTSTV YMELSSLRSED TAVYYCARGGYQLPHGRARAFDMWGQGT MVTVSS
SEQ ID NO: 63	α hCCR2 42G7 VL	AIRMTQSPLSLPVT LGQPASISCTSSQSLVYRDGTTYLNWFQ QRPGQSPRRLIYKVSNRDSGVPDRFTGSGSGTTFTLTISRVE AEDVGIYYCMQGT HWPLTFGQGTKVEIK
SEQ ID NO: 64	α hCCR2 43G12 VH	EVQLVESGGGLVQP GGSRLSCVASGFTFSDYWMSWVRQA PGKGLEWVANIKKDGSVNYYVDSVKGRFTISRDN AKNSLY LQMNSLR AEDTAVYYCTRFDYWGQGTLVTVSS
SEQ ID NO: 65	α hCCR2 43G12 VL	QAGLTQPPSVSKGLRQTATLTCTGNSNNVGNQGAAWLQQH QGHPPKLLFYRNNNRASGISERLSASRSGNTASLTITGLQPE DEADYYCLTW DSSLSVVVFGGGGTKLTVL
SEQ ID NO: 66	α hCSF1R emactuzumab VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDISWVRQA PGQGLEWMGV IWT DGGTNYAQKLQGRVTMTTDTSTSTAY MELRSLRSDDTAVYYCARDQRLYFDVWGQGTTVTVSS
SEQ ID NO: 67	α hCSF1R emactuzumab VL	DIQMTQSPSSLSASVGDRVTITCRASEDVNTYVSWYQQKPG KAPKLLIYAASNRYTGVP SRFSGSGSGTDFTLTISSLQPEDFA TYYCQQSF SYPTFGQGTKLEIK
SEQ ID NO: 68	hFc Hole	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVCTLP PSREEMTKNQVSLSCAVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDS DGSFFLVSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 69	α hCSF1R cabiralizumab VH	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDNYMIWVRQA PGQGLEWMGDINPYNGGTTFNQKFKGRVTITADKSTSTAY MELSSLRSED TAVYYCARESPYFSNLYVMDYWQGQTLVTV SS
SEQ ID NO: 70	α hCSF1R cabiralizumab VL	EIVLTQSPATLSLSPGERATLSCKASQSV D YDGDNYMNWYQ QKPGQAPRLLIYAASNLESGIPARFSGSGSGTDFTLTISSELEPE DFAVYYCHLSNEDLSTFGGGGTKVEIK

Table 5. Protein sequences for full heavy and light chains.

Full length	Variable	Constant	Fc
SEQ ID NO: 71	SEQ ID NO: 44	SEQ ID NO: 46	SEQ ID NO: 52
SEQ ID NO: 72	SEQ ID NO: 45	SEQ ID NO: 47	
SEQ ID NO: 73	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 53
SEQ ID NO: 74	SEQ ID NO: 50	SEQ ID NO: 51	
SEQ ID NO: 75	SEQ ID NO: 54	SEQ ID NO: 55	SEQ ID NO: 56
SEQ ID NO: 76	SEQ ID NO: 57	SEQ ID NO: 58	
SEQ ID NO: 77	SEQ ID NO: 59	SEQ ID NO: 55	SEQ ID NO: 56
SEQ ID NO: 78	SEQ ID NO: 60	SEQ ID NO: 61	
SEQ ID NO: 79	SEQ ID NO: 62	SEQ ID NO: 55	SEQ ID NO: 56
SEQ ID NO: 80	SEQ ID NO: 63	SEQ ID NO: 58	
SEQ ID NO: 81	SEQ ID NO: 64	SEQ ID NO: 55	SEQ ID NO: 56
SEQ ID NO: 82	SEQ ID NO: 65	SEQ ID NO: 58	
SEQ ID NO: 83	SEQ ID NO: 66	SEQ ID NO: 55	SEQ ID NO: 68
SEQ ID NO: 84	SEQ ID NO: 67	SEQ ID NO: 58	
SEQ ID NO: 85	SEQ ID NO: 69	SEQ ID NO: 55	SEQ ID NO: 68
SEQ ID NO: 86	SEQ ID NO: 70	SEQ ID NO: 58	

Table 6. Amino acid sequences of the chains used to construct multispecific molecules.

SEQ ID NO	Amino Acid Sequence
SEQ ID NO: 71	QVQLQESGPGLVQPSQTLSTCTVSGFSLTDFSVHWVRQPPGKGLEWMGRI RSEGNTDYN SALKSRLSISRDT SKSQVFLKMNSLQTEDTAIFYCTRGDILGFG YWGQGVMVTVSSAQTTAPSVYPLAPGCGD TTSSVT LGCLVKGYFPEPVTV TWN SGALSSDVHTFPAVLQSGLYTLTSSVTSS TWPSQTVTCNV AHPASSTKV DKKVERTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVL MISLSPIVTCVVVDV SEDDPDVQISW FVNNVEVHTAQTQTHREDYNSTLRVVSALPIQH QDWMSG KEFKCKVNNKDL PAPIERTISKPKGSVRAPQVYVLP PCEEEMTKKQVTLWC MVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKN WVERNSYSCSVVHEGLHNHHTTKSFSRTPGK
SEQ ID NO: 72	DIVMTQSPLSVSVTPGESASISCRSSKSL LHFKGITFVYWYLQKPGQSPQLLIF RMSSLASGVPDRFSGSGSETDFTLKISRVEAEDVGTYYCGQLLENPYTFGAG TKLELKRADAAPT VSIFFPSMEQLTSGGATV VCFVNNFYPRDISVKWKIDGS EQRDGVLD SVTDQDSKDYSTYSMSSTLSLTKVEYERHNL YTCEVVHKTSSSP VVKSFNRNEC
SEQ ID NO: 73	QVQLQQSGAELVKPGSSVKISCKASGYTFTSNFMHWIKQQPGNGLEWIGWI YPGDGDTEYNQKFNGKATLTADKSSSTAYMQLSSLTSEDSAVYFCAVNYG GYVLDAWGQGASVT VSSAETTAPSVYPLAPGTALKSNSMVT LGCLVKGYF PEPVTVTWN SGALSSGVHTFPAVLQSGLYTLTSSVT VPSSTWSSQAVTCNVA HPASSTKV DKKIVPRECNTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVL MISLS PIVTCVVVDVSEDDPDVQISW FVNNVEVHTAQTQTHREDYNSTLRVVSALPI QH QDWMSGKEFKCKVNNKDL PAPIERTISKPKGSVRAPQVCVLP PEEEMT KKQVTLSCAVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSK LRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK
SEQ ID NO: 74	EIVLTQSPTTMAASPGEKVTITCRASSSTNYMSWYQQKSGASPKPWYETSK LASGVPDRFSGSGSGTSYSFTISSMETEDAATYYCHQWSSTPLTFGSGTKLEI KRADAAPT VSIFFPSMEQLTSGGATV VCFVNNFYPRDISVKWKIDGSEQRDG VLDSVTDQDSKDYSTYSMSSTLSLTKVEYERHNL YTCEVVHKTSSSPVVKSFN

	RNEC
SEQ ID NO: 75	EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMNWVRQAPGKGLEWVGR IRTKNNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTF YGNGVWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE EVTCTVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREEM TKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 76	DVVMTQSPLSLPVTLGQPASISCKSSQSLLDSDGKTFLNWFQQRPGQSPRRLI YLVSCLDSGVPDRFSGSGGTDFTLKISRVEAEDVGVYYCWQGTHFPYTFG QGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC
SEQ ID NO: 77	EVQLVQSGAEVKKPGASVKVSCKASGYTFTGYHMHWRQAPGQGLEWM GWINPNSGVTKYAQKFQGRVTMTRDTSINTAYMELSRLRFDDTDVYYCAT GGFGYWGEGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREEMT KNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 78	LPVLTQPPSVSKGLRQTATLTCTGNSNNVGNQGAAWLQQHQGQPPKLLSYR NHNRPSPGVSEFSPSRSGDTSSLTITGLQPEDEADYYCLAWDSSLRAVFVGT GTKLTVLGQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DGSPVKAGVETTKPSKQSNKYYAASSYLSLTPEQWKSHRSYSCQVTHEGST VEKTVAPTECS
SEQ ID NO: 79	QVQLVQSGAEVKKPGASVKVSCKASGYTFSSYYMHWVRQAPGQGLEWMG IINPSGGNTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDVAVYYCARGG YQLPHGRARAFDMWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 80	AIRMTQSPLSLPVTLGQPASISCTSSQSLVYRDGTTYLNWFQQRPGQSPRRLI YKVSNRDSGVPDRFTGSGSGTFTLTISRVEAEDVGIYYCMQGTHWPLTFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGEC
SEQ ID NO: 81	EVQLVESGGGLVQPGGSLRLSCVASGFTFSDYWMSWVRQAPGKGLEWVA NIKKDGSVNYYVDSVKGRFTISRDNANKSLYLQMNSLRAEDTAVYYCTRFD YWGQGTLLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT

	KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 82	QAGLTQPPSVSKGLRQTATLTCTGNSNNVGNQGAAWLQQHQGHPPKLLFY RNNNRASGISERLSASRSGNTASLTITGLQPEDEADYYCLTWDSLSVVVFG GGTKLTVLGQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK ADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGS TVEKTVAPTECS
SEQ ID NO: 83	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDISWVRQAPGQGLEWMGV IWT DGGTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDQR LYFDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSREEMT KNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 84	DIQMTQSPSSLSASVGDRVTITCRASEDVENTYVSWYQQKPGKAPKLLIYAAS NRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSFSYPTFGQGTKLEIK RTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC
SEQ ID NO: 85	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDNYMIWVRQAPGQGLEWMG DINPYNGGTTFNQKFGRVTITADKSTSTAYMELSSLRSED TAVYYCARESP YFSNLYVMDYWGGGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPS REEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL VSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 86	EIVLTQSPATLSLSPGERATLSCKASQSVSDYDGDNYMNWYQQKPGQAPRLL IYAASNLESGIPARFSGSGSGTDFTLTISLEPEDFAVYYCHLSNEDLSTFGGG TKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC

Table 7. Sequences used to generate α CCR2/ α CSF1R multispecific molecules.

Multispecific Molecule	Heavy Chain 1	Light Chain 1	Heavy Chain 2	Light Chain 2
1	SEQ ID NO: 71	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 74
2	SEQ ID NO: 75	SEQ ID NO: 76	SEQ ID NO: 83	SEQ ID NO: 84
3	SEQ ID NO: 75	SEQ ID NO: 76	SEQ ID NO: 85	SEQ ID NO: 86
4	SEQ ID NO: 77	SEQ ID NO: 78	SEQ ID NO: 83	SEQ ID NO: 84
5	SEQ ID NO: 77	SEQ ID NO: 78	SEQ ID NO: 85	SEQ ID NO: 86

6	SEQ ID NO: 79	SEQ ID NO: 80	SEQ ID NO: 83	SEQ ID NO: 84
7	SEQ ID NO: 79	SEQ ID NO: 80	SEQ ID NO: 85	SEQ ID NO: 86
8	SEQ ID NO: 81	SEQ ID NO: 82	SEQ ID NO: 83	SEQ ID NO: 84
9	SEQ ID NO: 81	SEQ ID NO: 82	SEQ ID NO: 85	SEQ ID NO: 86

Example 2. UniTI-01 binding to cells expressing mCCR2 alone, mCSF1R alone, or both mCCR2 and mCSF1R

In this and the next few examples, multispecific molecule #1 (also referred to as UniTI-01) shown in Table 7 was characterized. UniTI-01 is an anti-CCR2/anti-CSF1R bispecific antibody. The variable region and full length sequences of UniTI-01 are also provided in Table 11. In a few examples, the bispecific antibody UniTI-01 was compared against an anti-CCR2 bivalent monospecific antibody or an anti-CSF1R bivalent monospecific antibody.

Table 11. Sequences of an anti-CCR2/anti-CSF1R bispecific antibody molecule

SEQ ID NO	Description	Sequence
SEQ ID NO: 44	α CCR2 MC12 VH	QVQLQESGPGLVQPSQTLSTCTVSGFSLTDFSVHWVRQPPG KGLEWMGRIRSEGNTDYN SALKSRLSISRDTSKSQVFLKMNS LQTEDTAIFYCTRGDILGFGYWGQGVMVTVSS
SEQ ID NO: 45	α CCR2 MC12 VL	DIVMTQSPLSVSVTPGESASISCRSSKSLHFKGITFVYWYLQ KPGQSPQLLIFRMSSLASGVPDRFSGSGSETDFTLKISRVEAED VGTYYCGQLLENPYTFGAGTKLELK
SEQ ID NO: 71	Full length α CCR2 MC12 heavy chain	QVQLQESGPGLVQPSQTLSTCTVSGFSLTDFSVHWVRQPPG KGLEWMGRIRSEGNTDYN SALKSRLSISRDTSKSQVFLKMNS LQTEDTAIFYCTRGDILGFGYWGQGVMVTVSSAQTTPSVYP LAPGCGDTSSTVTGLCLVKGYFPEPVTVTWNSGALSSDVHT FPAVLQSGLYTLTSSVTSSTWPSQTVTCNVAHPASSTKVDKK VERRTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVT CVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLR VVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSV RAPQVYVLPCEEEMTKKQVTLWCMVTDMPEDIYVEWTN NGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYS CSVVHEGLHNHHTTKSFSRTPGK
SEQ ID NO: 72	Full length α CCR2 MC12 light chain	DIVMTQSPLSVSVTPGESASISCRSSKSLHFKGITFVYWYLQ KPGQSPQLLIFRMSSLASGVPDRFSGSGSETDFTLKISRVEAED VGTYYCGQLLENPYTFGAGTKLELKRADAAPTVSIFPPSMEQ LTSGGATVVCVFN NFYPRDISVKWKIDGSEQRDGVLDSDVD QDSKDSTYSMSSTLSLTKEVERHNLYTCEVVHKTSSSPVVK SFNRNEC
SEQ ID NO: 48	α mCSF1R VH	QVQLQQSGAELVKPGSSVKISCKASGYTFTSNFMHWIKQQPG NGLEWIGWIYPGDGDTEYNQKFNGKATLTADKSSSTAYMQL SSLTSEDSAVYFCAVNYGGYVLDAGWGQGASVTVSS
SEQ ID NO: 50	α mCSF1R VL	EIVLTQSPTTMAASPGEKVTITCRASSSTNYMSWYQQKSGAS PKPWIYETSKLASGVPDRFSGSGSGTSYSFTISSMETEDAATY YCHQWSSTPLTFGSGTKLEIK

SEQ ID NO: 73	Full length αmCSF1R heavy chain	QVQLQQSGAELVKPGSSVKISCKASGYTFTSNFMHWIKQQPG NGLEWIGWIYPGDGDTEYNQKFNGKATLTADKSSSTAYMQL SSLTSEDSAVYFCAVNYGGYVLDAWGQGASVTVSSAETTAP SVYPLAPGTALKSNSMVTLGCLVKGYFPEPVTVTWNSGALSS GVHTFPAVLQSGLYTLTSSVTVPSSTWSSQAVTCNVAHPASS TKVDKKIVPRECNTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDV LMISLSPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTH REDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLPAPIE RTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPE DIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKN WVERNSYSCSVVHEGLHNHHTTKSFSRTPGK
SEQ ID NO: 74	Full length αmCSF1R light chain	EIVLTQSPTTMAASPGEKVTITCRASSSTNYMSWYQQKSGAS PKPWIYETSKLASGVDPDRFSGSGSGTSYSFTISSMETEDAATY YCHQWSSTPLTFGSGTKLEIKRADAAPTVSIFPPSMEQLTSGG ATVVCVFNNFYPRDISVKWKIDGSEQRDGVLDSDVTDQDSKD STYSMSSTLSLTKVEYERHNLTYTCEVVHKTSSSPVVKSFNRN EC

ExpiCHO cells were transiently transfected with mouse CCR2, mouse CSF1R, or both mouse CCR2 and CSF1R, according to the manufacturer’s instructions. In brief, the transfections were performed with ExpiCHO cells at a cell density of 5.6-6.3 x 10⁶ cells/mL and at least 95 % viability. For each transfection, 25 µg of DNA was diluted with 1 mL of OptiPro SFM (Gibco 12309050) and filtered using spin-X centrifuge tube filters (Corning 8160). A solution of 920 µL of OptiPro and 80 µL of expifectamine was added to the filtered DNA, incubated for 1 minute at room temperature, and then added to the cells. On day 1 of the transfection, the cells were enhanced using 150 µL of ExpiCHO enhancer.

On day 2 of the transfection, the cells were washed with PBS containing 1 % BSA (Sigma) and used to set 96-well V-bottom plates (Biotix AP-0350-9CVS) with 100,000 cells/well. UniTI-01 was added to the cells in serial dilutions and incubated for 1 hour at 4 °C. The plates were washed twice with PBS containing 1 % BSA. The secondary antibody was a 1:500 dilution of goat anti-mouse Fc biotin antibody (Invitrogen Cat. No. 31805), and incubated with the cells for 45 minutes at 4 °C. The plates were washed twice with PBS containing 1 % BSA. For detection, 1.56 x 10⁻³ µg of streptavidin-PE (eBioscience Cat. No. 12-4317-87) was used, per well, and incubated for 1 hour at 4 °C. The plates were read on a CytoFLEX S (Beckman Coulter). Data were calculated as the median fluorescence intensity of the PE-positive population vs. the median fluorescence intensity of the PE-negative population. The data was normalized for the percent of total median fluorescence intensity.

Without wishing to be bound by theory, UniTI-01 may preferentially bind cells expressing both CCR2 and CSF1R relative to cells that express either CCR2 or CSF1R. Consistent with the hypothesis that dual target binding increases the avidity of UniTI-01 for the target cell, UniTI-01 exhibited enhanced binding to CCR2 and CSF1R double positive cells, relative to single positive cells (FIG. 1). UniTI-01 showed binding with an EC₅₀ of 15 nM to cells expressing only CCR2. For cells expressing only CSF1R, UniTI-01 had an EC₅₀ of 1 nM. UniTI-01 displayed an EC₅₀ of 400 pM to cells that expressed both CCR2 and CSF1R.

Example 3. UniTI-01 inhibits MCP1-induced migration of bone marrow derived monocytes in vitro

Mouse bone marrow cells were isolated from femurs of healthy Balb/c mice and differentiated into monocytes in the presence of mCSF1 for four days. Monocyte differentiation was assessed by flow-cytometric analysis of CCR2 and CSF1R expression on hematopoietic cells. The differentiated cells were counted, and cultured with different concentrations of Isotype (mIgG2a), anti-CCR2 and UniTI-01 respectively for 30 minutes at 37°C. Antibody treated cells were subsequently added on the upper chamber in the transwell insert plates, which contained MCP1 (CCL2) in the bottom chamber. MCP1 induced migration was assessed after collecting the media from bottom chamber of transwell plates and cell number enumeration was performed by flow-cytometric analysis.

The anti-CCR2/anti-CSF1R bispecific antibody UniTI-01 inhibited the MCP1 induced migration of monocytes across the transwell in a dose dependent manner (FIG. 2). At doses 3.7μM and above, monocyte migration was reduced to the levels observed in the absence of chemokine (FIG. 2). Similar results were obtained with anti-CCR2 antibody treatment, while treatment of cells with IgG2a did not influence migration (FIG. 2).

Example 4. UniTI-01 inhibits mCSF-1-dependent proliferation of bone marrow-derived macrophages in vitro

Mouse bone marrow cells were isolated from femurs of healthy Balb/c mice and differentiated into monocytes in the presence of mCSF1 for four days. Flow cytometry analysis showed bone marrow cells did not appreciably express CCR2 (FIG. 3A; left panel) at day 0. However, after 4 days of cell culture in the presence of mCSF1, a significant portion of bone

marrow cell differentiated into monocytes which displayed CCR2 and CSF1R expression (FIG. 3A; right panel). Monocytes were counted and plated in 96 well plates in the presence of mCSF1. UniTI-01, anti-CSF1R or mIgG2a antibody was added to the cells containing mCSF1. After 72 hours of incubation at 37°C, cell proliferation metabolic activity was assessed by colorimetric reading at OD570 following manufacturer's protocol for the MTT assay kit.

mCSF1 induced differentiation of monocytes to macrophages, visualized as long fibroblastic cells under the microscope. UniTI-01 and anti-CSF1R antibodies prevented the proliferation of macrophages in the presence of mCSF1 (FIG. 3B).

Example 5. UniTI-01 does not inhibit mCSF-1-dependent bone marrow-derived monocyte differentiation in vitro

Bone marrow cells were extracted from both femur and tibia of a naive (non-tumor bearing) Balb/c mouse. UniTI-01, anti-CSF1R or mIgG2 antibodies were pre-incubated with freshly isolated bone marrow cells for 30 minutes at 37°C before the addition of mCSF-1 to allow monocyte differentiation. After 4 days of incubation at 37°C, cells were collected for flow cytometry staining to identify differentiated monocytes. Cells were stained with fluorescent-labeled antibodies for 15 minutes at 4°C followed by flow cytometry analysis. Monocytes are gated as Live, CD45+, CD11b+, Ly6C+, Ly6G- cells.

Consistent with the observation that bone marrow precursor cells did not significantly express CCR2 at day 0 (FIG. 3A), the anti-CCR2/anti-CSF1R bispecific antibody UniTI-01 did not inhibit mCSF-1-dependent bone marrow-derived monocyte differentiation in vitro after 4 days of cell culture (FIG. 4). In contrast, the anti-CSF1R bivalent monospecific antibody significantly inhibited mCSF-1-dependent bone marrow-derived monocyte differentiation in vitro (FIG. 4).

Example 6. UniTI-01 specifically binds to primary intratumoral M-MDSCs and M2-like macrophages in vitro

LLC tumors, grown in B6- albino mice, were harvested at a volume of 500- 800mm³ and dissociated using liberase DL + Dnase I for 30minutes at 37°C, followed by the dissociation program m_imptumor_01 on the GentleMacs. Single cell suspensions were filtered through a 70µm strainer and total cells were stained with fluorescently labeled antibodies. For this binding

study, 100 μ g of UniTI-01 was labeled using the Alexa Fluor 647 Antibody Labeling Kit (ThermoFisher Scientific), the concentration of labeled antibody was determined by Nanodrop, and the indicated serial dilutions (5 μ M, 1 μ M, 0.1 μ M, 0.01 μ M) were made in FACS buffer. M-MDSCs were gated by live CD45+CD11b+Ly6ChighLy6G-, M2 macrophages were gated by live CD45+CD11b+F4/80+CD206+, CD3+ T cells were gated by live CD45+CD3+, and neutrophils were gated by live CD45+CD11b+Ly6G+. The cells were stained for 15 minutes on ice in the dark, stained with zombie violet for viability, and immediately acquired on the cytometer.

Concentration-dependent binding of UniTI-01 to M2 macrophages and M-MDSCs is shown in FIG. 5.

Example 7. UniTI-01 depletes suppressive myeloid cells in several mouse models in vivo

Mice were injected either with MC38 colon cancer cell line (B6 albino mice) or EMT6 breast cancer cell line (BALB/c mice) and once tumors reached a volume of 150-200mm³, the same mice were randomized and grouped into two arms. One arm received a treatment of 20mg/kg UniTI-01 via ip route at a dose of 20mg/kg on day 1, 4, 7 and 10 and the other received PBS (vehicle) at the same schedule. Twenty-four hours after the 4th dose, tumors were harvested for flow cytometry analysis. Tumors were minced into ~2mm pieces and dissociated with liberase + dnase I for 30 minutes at 37°C, followed by using a 1 minute tumor blend program on the gentleMACs. Single cell suspensions were made by filtering through a 70 μ M filter and counted. Cells were then stained for flow cytometry analysis. TAMs were gated by live CD45+CD11b+Ly6G-Ly6C-F4/80+ and M-MDSCs were gated by live CD45+CD11b+Ly6G-Ly6C^{high}. Each point represents a single mouse. Error bars represent the mean and standard error between individual mice. Statistics were calculated using Student's t test.

Consistent with the binding to M2 macrophages and M-MDSCs observed in Example 6, the anti-CCR2/anti-CSF1R bispecific antibody UniTI-01 reduced TAMs and M-MDSCs in both EMT6 and MC38 syngeneic tumor models (FIG. 6).

Example 8. UniTI-01 depletes tumor-associated macrophages but spares Kupffer cells in vivo

Balb/c mice were injected with EMT6 syngeneic breast cell line and once tumors reached a volume of 150-200mm³, were randomized and grouped into three arms. One arm received a treatment of 20mg/kg UniTI-01 via ip route at a dose of 20mg/kg, the second arm received a treatment of 10mg/kg anti-CSF1R antibody and the third arm received PBS on day 1, 4, 7 and 10. Twenty-four hours after the 4th dose, mice were sacrificed and tumors and livers were harvested and formalin fixed and paraffin embedded. To detect the macrophage populations in liver and tumors, tissue sections were immunohistochemically stained with F4/80 antibody (Cell Signaling) and detected by Envision system. Approximately 10 regions of interest per tumor or liver section were analyzed by ImageJ software.

Without wishing to be bound by theory, UniTI-01 may preferentially bind to cells expressing both CCR2 and CSF1R relative to cells expressing either CCR2 or CSF1R, and may have less an effect on tissue-resident macrophages, such as the liver-resident Kupffer cells, which do not express CCR2, relative to tumor-associated macrophages which express both CCR2 and CSF1R.

Consistent with the data described earlier (FIG. 6), the anti-CCR2/anti-CSF1R bispecific antibody UniTI-01 markedly depleted tumor-associated macrophages (FIGs. 7A and 7C). In contrast, UniTI-01 did not appreciably deplete tissue-resident macrophages in the liver (FIGs. 7B and 7D), whereas the anti-CSF1R bivalent monospecific antibody significantly reduced macrophages in both compartments (FIGs. 7A-7D).

Example 9. UniTI-01 does not inhibit CSF-1 dependent cell survival in CCR2-negative NFS-60 cells in vitro

CCR2-negative, CSF1R-positive NFS-60 cells were cultured in phenol-red-free media in the presence of UniTI-01, a monovalent monospecific anti-CSF1R antibody, a bivalent monospecific anti-CSF1R antibody, or a mIgG2 antibody for 30 minutes at 37°C followed by the addition of mCSF-1 for cell survival. After 48 hours of incubation at 37°C, cell viability metabolic activity was assessed by colorimetric reading at OD570 following manufacturer's protocol for the MTT assay kit.

As shown in FIG. 8A, the anti-CSF1R bivalent monospecific antibody (aCSF1R) markedly reduced viability of NFS-60 cells at high antibody concentrations. In contrast, the anti-CCR2/anti-CSF1R bispecific antibody UniTI-01 or the monovalent monospecific anti-CSF1R antibody (mono-aCSF1R) did not inhibit CSF-1 dependent cell survival in CCR2-negative NFS-60 cells in vitro (FIG. 8A). Without wishing to be bound by theory, this data suggests that the anti-CCR2/anti-CSF1R bispecific antibody UniTI-01 may be less likely to inhibit CCR2-negative, CSF1R-positive cells, compared with an anti-CSF1R bivalent monospecific antibody, which is consistent with the results described in FIGs. 7B and 7D. To confirm NFS-60 did not express CCR2, cells were washed with BSA containing PBS buffer and fluorescent-labeled antibodies for CSF1R and CCR2 for 20 minutes at 4°C followed by flow cytometry analysis. As shown in FIG. 8B, NFS-60 expressed CSF1R but not CCR2.

Example 10. UniTI-01 promotes CD8+ T cell infiltration in EMT6 tumors in vivo

Balb/c mice were injected with EMT6 syngeneic breast cell line and once tumors reached a volume of 150-200mm³, were randomized and grouped into three arms. One arm received a treatment of 20mg/kg UniTI-01 via ip route at a dose of 20mg/kg, the second arm received a treatment of 10mg/kg anti-PDL1 and the third arm received PBS on day 1, 4, 7 and 10. Twenty-four hours after the 4th dose, mice were sacrificed and tumors were harvested for flow cytometry analysis. Tumors were minced into ~2mm pieces and dissociated with liberase + dnase I for 30 minutes at 37°C, followed by using a 1 minute tumor blend program on the gentleMACS. Single cell suspensions were made by filtering through a 70µM filter and counted. Cells were then stained for flow cytometry analysis. CD8 T cells were gated by staining on live CD8 cells that expressed CD45 and CD3. Each point represents a single mouse tumor. Error bars represent the mean and standard error between individual mice. Statistics were calculated using one way ANOVA analysis.

The anti-CCR2/anti-CSF1R bispecific antibody UniTI-01 significantly increased CD8+ T cell infiltration in EMT6 tumors in vivo (FIG. 9).

Example 11. UniTI-01 reduces Treg frequency and increases CD8 T cells/Treg ratio in EMT6 tumors in vivo

B6 albino mice were injected with MC38 syngeneic colon cell line and once tumors reached a volume of 150-200mm³, were randomized and grouped into three arms. One arm received a treatment of 20mg/kg UniTI-01 via ip route at a dose of 20mg/kg, the second arm received a treatment of 10mg/kg anti-PDL1 and the third arm received PBS on day 1, 4, 7 and 10. Twenty-four hours after the 4th dose, mice were sacrificed and tumors were harvested for immune profiling by Flow cytometry. Tumors were minced into ~2mm pieces and dissociated with liberase + dnase I for 30 minutes at 37°C, followed by using a 1 minute tumor blend program on the gentleMACs. Single cell suspensions were made by filtering through a 70µM filter and counted. Cells were then stained for flow cytometry analysis. T regulatory cells were analyzed by gating on live CD4+ Foxp3+ T cells. In addition, CD8 T cells were gated by staining on live CD8 cells that expressed CD45 and CD3. Each point represents a single mouse tumor. Error bars represent the mean and standard error between individual mice. Statistics were calculated using one way ANOVA analysis.

Treatment with UniTI-01 led to a greater reduction in Treg frequency and a greater increase in the CD8+ T cell/Treg ratio in the tumor, compared with treatment with the anti-PDL1 antibody (FIGs. 10A and 10B).

Example 12. UniTI-01 shows antitumor efficacy, tumor regressions and enhanced survival when used in combination with anti-PDL1 antibody

Tumor growth inhibition of UniTI-01 in combination with anti-PDL1 antibody was tested in a subcutaneous mouse syngeneic EMT6 breast cancer model. ~8 week old female BALB/c (Jackson Labs) were acclimatized for 3 days prior to start of the studies. Mice were housed 5 animals per cage, and the disposable cages were placed in Innovive IVC mouse racks. EMT6 cells previously tested to be free from mouse pathogens (mouse CLEAR panel, Charles River Labs) were implanted subcutaneously on day 0 at a density of 0.5 x 10⁶ cells in the right flanks of BALB/c mice. Tumors were measured and recorded in two dimensions twice weekly using a digital caliper. Tumor volumes (mm³) were calculated using the formula width x width x length x 0.52. Following tumor volume measurements on day 7 post implantation, mice were randomized and grouped into four arms according to a mean tumor volume of 77mm³. One arm

was treated with PBS, the second arm was treated with anti-PDL1 antibody at a dose of 10mg/kg, the third arm treated with UniTI-01 at a dose of 20 mg/kg, and the fourth arm treated with a combination of anti-PDL1 antibody and UniTI-01. All treatments were via ip route and the schedules were twice weekly for up to four weeks of dosing. All the agents were formulated freshly in PBS prior to dosing. Tumor volume measurements continued up to 80 days. Tumor volume data is plotted as Mean $\pm$ SEM. In addition, survival is recorded based on the time to reach the study endpoint of a TV of 2000mm³ and plotted as a Kaplan Meier curve. Statistics were determined by Log- rank test and p- values are plotted.

As shown in FIGs. 11A and 11B, treatment with UniTI-01 improved the survival of tumor-bearing animals. Combining UniTI-01 and an anti-PDL1 antibody further improved the antitumor efficacy, tumor regressions and survival in vivo (FIGs. 11A and 11B).

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. An isolated multispecific, e.g., a bispecific, molecule, comprising:
 - (i) a first immunosuppressive myeloid cell (IMC) binding moiety (e.g., a first tumor associated macrophage (TAM) binding moiety; or a first myeloid derived suppressor cell (MDSC) binding moiety) (e.g., an antibody molecule); and
 - (ii) a second IMC binding moiety (e.g., a second TAM binding moiety; or a second MDSC binding moiety) (e.g., an antibody molecule), wherein the first and the second IMC (e.g., TAM or MDSC) binding moieties are different, optionally wherein the first and the second IMC (e.g., TAM or MDSC) binding moieties bind to different epitopes, optionally wherein the first and the second IMC (e.g., TAM or MDSC) binding moieties bind to different antigens.
2. The multispecific molecule of claim 1, wherein the first IMC binding moiety is a first MDSC binding moiety; and the second IMC binding moiety is a second MDSC binding moiety.
3. The multispecific molecule of claim 1, wherein the first IMC binding moiety is a first TAM binding moiety; and the second IMC binding moiety is a second TAM binding moiety.
4. The multispecific molecule of claim 3, wherein the first TAM binding moiety binds to CSF1R, CCR2, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1; and the second TAM binding moiety binds to CCR2, CSF1R, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1.
5. The multispecific molecule of claim 4, wherein the first TAM binding moiety binds to CSF1R, CCR2, CXCR2, or PD-L1 (e.g., human CSF1R, CCR2, CXCR2, or PD-L1) and the second TAM binding moiety binds to CCR2, CSF1R, CXCR2, or PD-L1 (e.g., human CCR2, CSF1R, CXCR2, or PD-L1).
6. The multispecific molecule of claim 4, wherein the first TAM binding moiety binds to CSF1R and the second TAM binding moiety binds to CCR2.

7. The multispecific molecule of claim 4, wherein the first TAM binding moiety binds to CSF1R and the second TAM binding moiety binds to CXCR2.

8. The multispecific molecule of claim 4, wherein the first TAM binding moiety binds to CCR2 and the second TAM binding moiety binds to CXCR2.

9. The multispecific molecule of claim 4, wherein the first TAM binding moiety binds to CSF1R and the second TAM binding moiety binds to PD-L1.

10. The multispecific molecule of any one of claims 3-9, wherein the first TAM binding moiety binds to CSF1R, CCR2, CXCR2, or PD-L1 with a dissociation constant of less than about 10 nM, and more typically, 10 – 100 pM; and the second TAM binding moiety binds to CCR2, CSF1R, CXCR2, or PD-L1 with a dissociation constant of less than about 10 nM, and more typically, 10 – 100 pM.

11. The multispecific molecule of any one of claims 3-10, wherein the first TAM binding moiety binds to a conformational or a linear epitope on CSF1R, CCR2, CXCR2, or PD-L1; and the second TAM binding moiety binds to a conformational or a linear epitope on CCR2, CSF1R, CXCR2, or PD-L1.

12. The multispecific molecule of any one of claims 1-11, wherein the multispecific molecule comprises at least two non-contiguous polypeptide chains.

13. The multispecific molecule of any one of claims 1-12, wherein the first IMC binding moiety comprises a first anti-IMC antibody molecule and/or the second IMC binding moiety comprises a second anti-IMC antibody molecule.

14. The multispecific molecule of claim 13, wherein the first anti-IMC antibody molecule and the second anti-IMC antibody molecule are, independently, a full antibody (e.g., an antibody that includes at least one, and preferably two, complete heavy chains, and at least one,

and preferably two, complete light chains), or an antigen-binding fragment (e.g., a Fab, F(ab')₂, Fv, a scFv, a single domain antibody, or a diabody (dAb)).

15. The multispecific molecule of claim 13 or 14, wherein the first anti-IMC antibody molecule and/or the second anti-IMC antibody molecule comprises a heavy chain constant region chosen from IgG1, IgG2, IgG3, or IgG4, or a fragment thereof.

16. The multispecific molecule of any one of claims 13-15, wherein the first anti-IMC antibody molecule and/or the second anti-IMC antibody molecule comprises a light chain constant region chosen from the light chain constant regions of kappa or lambda, or a fragment thereof.

17. The multispecific molecule of any one of claims 13-15, wherein the first anti-IMC antibody molecule comprises a kappa light chain constant region, or a fragment thereof, and the second anti-IMC antibody molecule comprises a lambda light chain constant region, or a fragment thereof.

18. The multispecific molecule of any one of claims 13-15, wherein the first anti-IMC antibody molecule comprises a lambda light chain constant region, or a fragment thereof, and the second anti-IMC antibody molecule comprises a kappa light chain constant region, or a fragment thereof.

19. The multispecific molecule of any one of claims 13-15, wherein the first anti-IMC antibody molecule and the second anti-IMC antibody molecule have a common light chain variable region.

20. The multispecific molecule of any one of claims 1-19, further comprising a heavy chain constant region (e.g., an Fc region) chosen from the heavy chain constant regions of IgG1, IgG2, and IgG4, more particularly, the heavy chain constant region of human IgG1, IgG2 or IgG4.

21. The multispecific molecule of claim 20, wherein the heavy chain constant region (e.g., an Fc region) is linked to, e.g., covalently linked to, one or both of the first anti-IMC antibody molecule and the second anti-IMC antibody molecule.

22. The multispecific molecule of claim 20 or 21, wherein the heavy chain constant region (e.g., an Fc region) comprises one or more mutations that increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function, relative to a naturally-existing heavy chain constant region.

23. The multispecific molecule of any one of claims 13-22, wherein the first anti-IMC antibody molecule comprises a first heavy chain constant region (e.g., a first Fc region) and the second anti-IMC antibody molecule comprises a second heavy chain constant region (e.g., a second Fc region), wherein the first heavy chain constant region comprises one or more mutations that increase heterodimerization of the first heavy chain constant region and the second heavy chain constant region, relative to a naturally-existing heavy chain constant region, and/or wherein the second heavy chain constant region comprises one or more mutations that increase heterodimerization of the second heavy chain constant region and the first heavy chain constant region, relative to a naturally-existing heavy chain constant region.

24. The multispecific molecule of claim 23, wherein the first and the second heavy chain constant regions (e.g., first and second Fc regions) comprise one or more of: a paired cavity-protuberance (“knob-in-a hole”), an electrostatic interaction, or a strand-exchange, such that a greater ratio of heteromultimer:homomultimer forms, e.g., relative to naturally-existing heavy chain constant regions.

25. The multispecific molecule of claim 23 or 24, wherein the first and/or second heavy chain constant region (e.g., a first and/or second Fc region, e.g., a first and/or second IgG1 Fc region) comprises an amino acid substitution at a position chosen from one or more of 347, 349, 350, 351, 366, 368, 370, 392, 394, 395, 397, 398, 399, 405, 407, or 409, numbered based on the Eu numbering system.

26. The multispecific molecule of any one of claims 23-25, wherein the first and/or second heavy chain constant region (e.g., a first and/or second Fc region, e.g., a first and/or second IgG1 Fc region) comprises an amino acid substitution chosen from: T366S, L368A, Y407V, or Y349C (e.g., corresponding to a cavity or hole), or T366W or S354C (e.g., corresponding to a protuberance or knob), or a combination thereof, numbered based on the Eu numbering system.

27. The multispecific molecule of any one of claims 1-26, further comprising a linker, e.g., a linker between one or more of: the first anti-IMC antibody molecule and the second anti-IMC antibody molecule, the first anti-IMC antibody molecule and the heavy chain constant region (e.g., the Fc region), or the second anti-IMC antibody molecule and the heavy chain constant region (e.g., the Fc region).

28. The multispecific molecule of claim 27, wherein the linker is chosen from: a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker.

29. The multispecific molecule of claim 28, wherein the linker is a peptide linker.

30. The multispecific molecule of claim 29, wherein the peptide linker comprises Gly and Ser.

31. The multispecific molecule of any one of claim 20-30, wherein the heavy chain constant region (e.g., Fc region) induces antibody dependent cellular cytotoxicity (ADCC).

32. The multispecific molecule of any one of claims 1-31, wherein the first or the second IMC binding moiety is an antibody molecule that binds to CSF1R and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 48, SEQ ID NO: 66, or SEQ ID NO: 69, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or

insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 48, SEQ ID NO: 66, or SEQ ID NO: 69; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 50, SEQ ID NO: 67, or SEQ ID NO: 70, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 50, SEQ ID NO: 67, or SEQ ID NO: 70.

33. The multispecific molecule of claim 32, wherein the antibody molecule that binds to CSF1R comprises the heavy chain variable region sequence of: SEQ ID NO: 48, SEQ ID NO: 66, or SEQ ID NO: 69, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 48, SEQ ID NO: 66, or SEQ ID NO: 69); and/or comprises the light chain variable region sequence of: SEQ ID NO: 50, SEQ ID NO: 67, or SEQ ID NO: 70, or an amino acid sequence substantially identical thereto (*e.g.*, 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 50, SEQ ID NO: 67, or SEQ ID NO: 70).

34. The multispecific molecule of any one of claims 1-33, wherein the first or the second IMC binding moiety is an antibody molecule that binds to CCR2 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 64, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 64; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO: 63, SEQ ID NO: 65, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO: 63, SEQ ID NO: 65.

35. The multispecific molecule of claim 34, wherein the antibody molecule that binds to CCR2 comprises the heavy chain variable region sequence of: SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 64, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 64); and/or comprises the light chain variable region sequence of: SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO: 63, SEQ ID NO: 65, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) to the amino acid sequence of SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO: 63, SEQ ID NO: 65).

36. The multispecific molecule of any one of claims 1-33, wherein the first or the second IMC binding moiety is an antibody molecule that binds to PD-L1 and comprises one, two, or three CDRs from the heavy chain variable region sequence of: SEQ ID NO: 109, SEQ ID NO: 111, or SEQ ID NO: 113, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 109, SEQ ID NO: 111, or SEQ ID NO: 113; and/or comprises one, two, or three CDRs from the light chain variable region sequence of: SEQ ID NO: 110, SEQ ID NO: 112, or SEQ ID NO: 114, or a closely related CDR, *e.g.*, CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (*e.g.*, substitutions, deletions, or insertions, *e.g.*, conservative substitutions) from a CDR of SEQ ID NO: 110, SEQ ID NO: 112, or SEQ ID NO: 114.

37. The multispecific molecule of claim 36, wherein the antibody molecule that binds to PD-L1 comprises the heavy chain variable region sequence of: SEQ ID NO: 109, SEQ ID NO: 111, or SEQ ID NO: 113, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten

or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 109, SEQ ID NO: 111, or SEQ ID NO: 113); and/or comprises the light chain variable region sequence of: SEQ ID NO: 110, SEQ ID NO: 112, or SEQ ID NO: 114, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 110, SEQ ID NO: 112, or SEQ ID NO: 114).

38. The multispecific molecule of any one of claims 1-37, wherein:

(i) the first IMC binding moiety binds to a first antigen (e.g., CSF1R, CCR2, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1) monovalently, and/or

(ii) the second IMC binding moiety binds to a second antigen (e.g., CCR2, CSF1R, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1) monovalently, wherein:

the first antigen is different from the second antigen.

39. The multispecific molecule of any one of claims 1-38, wherein:

(i) the multispecific molecule binds to a first antigen (e.g., CSF1R, CCR2, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1) monovalently, and/or

(ii) the multispecific molecule binds to a second antigen (e.g., CCR2, CSF1R, CXCR2, CD86, CD163, CX3CR1, MARCO, CD204, CD52, folate receptor beta, or PD-L1) monovalently, wherein:

the first antigen is different from the second antigen.

40. The multispecific molecule of any one of claims 1-39, wherein:

(i) the multispecific molecule inhibits a first antigen in the presence of a second antigen, optionally wherein the multispecific molecule reduces an activity of the first antigen in a cell, e.g., by at least 40, 50, 60, 70, 80, or 90%, when the cell expresses both the first antigen and the second antigen on the cell surface, and/or

(ii) the multispecific molecule does not inhibit or does not substantially inhibit the first antigen in the absence of the second antigen, optionally wherein the multispecific molecule does not reduce an activity of the first antigen, or does not reduce an activity of the first antigen by more than 2, 4, 6, 8, 10, or 15%, when the cell expresses the first antigen but not the second antigen on the cell surface.

41. The multispecific molecule of any one of claims 1-40, wherein:

(i) the multispecific molecule inhibits a second antigen in the presence of a first antigen, optionally wherein the multispecific molecule reduces an activity of the second antigen in a cell, e.g., by at least 40, 50, 60, 70, 80, or 90%, when the cell expresses both the first antigen and the second antigen on the cell surface, and/or

(ii) the multispecific molecule does not inhibit or does not substantially inhibit the second antigen in the absence of the first antigen, optionally wherein the multispecific molecule does not reduce an activity of the second antigen, or does not reduce an activity of the second antigen by more than 2, 4, 6, 8, 10, or 15%, when the cell expresses the second antigen but not the first antigen on the cell surface.

42. The multispecific molecule of any one of claims 1-41, comprising one or more additional binding moieties (e.g., a third binding moiety, a fourth binding moiety, (e.g., a trispecific or a tetraspecific molecule).

43. The multispecific molecule of any one of claims 3-42, comprising a third TAM binding moiety (e.g., an antibody molecule).

44. The multispecific molecule of claim 43, wherein the third TAM binding moiety is different from the first and the second TAM binding moieties.

45. The multispecific molecule of claim 43, wherein the first TAM binding moiety binds to human CSF1R, the second TAM binding moiety binds to human CCR2, and the third TAM binding moiety binds to CXCR2.

46. The multispecific molecule of claim 42, wherein the multispecific molecule comprises a third binding moiety (e.g., antibody molecule) that is a tumor targeting moiety.

47. The multispecific molecule of claim 46, wherein the tumor targeting moiety binds to PD-L1, mesothelin, CD47, ganglioside 2 (GD2), prostate stem cell antigen (PSCA), prostate specific membrane antigen (PMSA), prostate-specific antigen (PSA), carcinoembryonic antigen (CEA), Ron Kinase, c-Met, Immature laminin receptor, TAG-72, BING-4, Calcium-activated chloride channel 2, Cyclin-B1, 9D7, Ep-CAM, EphA3, Her2/neu, Telomerase, SAP-1, Survivin, NY-ESO-1/LAGE-1, PRAME, SSX-2, Melan-A/MART-1, Gp100/pmel17, Tyrosinase, TRP-1/-2, MC1R, β -catenin, BRCA1/2, CDK4, CML66, Fibronectin, p53, Ras, TGF-B receptor, AFP, ETA, MAGE, MUC-1, CA-125, BAGE, GAGE, NY-ESO-1, β -catenin, CDK4, CDC27, CD47, α actinin-4, TRP1/gp75, TRP2, gp100, Melan-A/MART1, gangliosides, WT1, EphA3, Epidermal growth factor receptor (EGFR), CD20, MART-2, MART-1, MUC1, MUC2, MUM1, MUM2, MUM3, NA88-1, NPM, OA1, OGT, RCC, RUI1, RUI2, SAGE, TRG, TRP1, TSTA, Folate receptor alpha, L1-CAM, CAIX, EGFRvIII, gpA33, GD3, GM2, VEGFR, Integrins (Integrin α V β 3, Integrin α 5 β 1), Carbohydrates (Le), IGF1R, EPHA3, TRAILR1, TRAILR2, or RANKL.

48. The multispecific molecule of claim 4, which is a bispecific molecule, wherein:

(i) the first TAM binding moiety (e.g., a binding moiety that binds to a first TAM antigen, e.g., CSF1R, CCR2, CXCR2, or PD-L1) comprises a first and a second non-contiguous polypeptides, and

(ii) the second TAM binding moiety (e.g., a binding moiety that binds to a second TAM antigen, e.g., CCR2, CSF1R, CXCR2, or PD-L1) comprises a third and a fourth non-contiguous polypeptides, wherein:

(a) the first polypeptide comprises, e.g., in the N- to C-orientation, a first VH, a first CH1, connected, optionally via a linker, to a first domain (e.g., a first Fc region) that promotes association between the first and the third polypeptides,

(b) the second polypeptide comprises, e.g., in the N- to C-orientation, a first VL and a first CL,

(c) the third polypeptide comprises, e.g., in the N- to C-orientation, a second VH, a second CH1, connected, optionally via a linker, to a second domain (e.g., a second Fc region) that promotes association between the first and the third polypeptides, and

(d) the fourth polypeptide comprises, e.g., in the N- to C-orientation, a second VL and a second CL.

49. The multispecific molecule of claim 48, wherein the first and the second domains (e.g., the first and the second Fc regions) form a homo- or heterodimer.

50. An isolated multispecific, e.g., a bispecific, molecule, comprising:

- (i) an anti-CSF1R binding moiety (e.g., an anti-CSF1R antibody molecule); and
- (ii) an anti-CCR2 binding moiety (e.g., an anti-CCR2 antibody molecule).

51. The multispecific molecule of claim 50, wherein the multispecific molecule has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or more) of the following properties:

(i) the multispecific molecule preferentially binds to a CSF1R-positive, CCR2-positive cell relative to a CSF1R-positive, CCR2-negative cell, or a CSF1R-negative, CCR2-positive cell, e.g., the binding of the multispecific molecule to the CSF1R-positive, CCR2-positive cell is at least 2, 4, 6, 8, 10, 15, 20, or 25-fold stronger than the binding of the multispecific molecule to the CSF1R-positive, CCR2-negative cell, or the CSF1R-negative, CCR2-positive cell, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 2 with respect to FIG. 1;

(ii) the multispecific molecule preferentially binds to a CSF1R-positive, CCR2-positive cell relative to a CSF1R-positive, CCR2-negative cell, e.g., the EC50 of the multispecific molecule for binding to a CSF1R-positive, CCR2-positive cell is no more than 60, 50, 40, 30, 20, or 10 % of the EC50 of the multispecific molecule for binding to a CSF1R-positive, CCR2-negative cell, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 2 with respect to FIG. 1;

(iii) the multispecific molecule preferentially binds to a CSF1R-positive, CCR2-positive cell relative to a CSF1R-negative, CCR2-positive cell, e.g., the EC50 of the multispecific molecule for binding to a CSF1R-positive, CCR2-positive cell is no more than 50, 40, 30, 20, 10,

or 5% of the EC50 of the multispecific molecule for binding to a CSF1R-negative, CCR2-positive cell, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 2 with respect to FIG. 1;

(iv) the multispecific molecule preferentially binds to tumor-associated macrophages (TAMs) or myeloid derived suppressor cells (MDSCs) relative to T cells, NK cells, neutrophils, tissue-resident macrophages (e.g., Kupffer cells), or Langerhans cells, e.g., the binding of the multispecific molecule to TAMs or MDSCs is at least 2, 4, 6, 8, 10, 15, 20, or 25-fold stronger than the binding of the multispecific molecule to T cells, NK cells, neutrophils, tissue-resident macrophages (e.g., Kupffer cells), or Langerhans cells, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 6 with respect to FIG. 5;

(v) the multispecific molecule inhibits monocyte migration, e.g., monocyte chemoattractant protein 1 (MCP1)-induced monocyte migration, e.g., reduces MCP1-induced monocyte migration by at least 40, 50, 60, or 70%, e.g., as measured using a transwell plate migration assay, e.g., as measured using methods described in Example 3 with respect to FIG. 2;

(vi) the multispecific molecule inhibits the proliferation of macrophages, e.g., bone marrow-derived macrophages, e.g., CSF-1-induced proliferation of bone marrow-derived macrophages, e.g., reduces CSF-1-induced proliferation of bone marrow-derived macrophages by at least 50, 60, 70, or 80%, e.g., as measured using a cell proliferation MTT assay, e.g., as measured using methods described in Example 4 with respect to FIG. 3B;

(vii) the multispecific molecule does not inhibit or does not substantially inhibit the differentiation of monocytes, e.g., bone marrow-derived monocytes, e.g., CSF-1-induced differentiation of bone marrow-derived monocytes, e.g., does not reduce CSF-1-induced differentiation of bone marrow-derived monocytes by more than 2, 4, 6, 8, or 10%, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 5 with respect to FIG. 4;

(viii) the multispecific molecule depletes suppressive myeloid cells, e.g., TAMs or MDSCs, e.g., reduces the number of suppressive myeloid cells, e.g., TAMs or MDSCs, by at least 80, 85, 90, 95, 99, or 99.5%, in vivo, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 7 with respect to FIG. 6;

(ix) the multispecific molecule does not deplete or does not substantially deplete tissue-resident macrophages, e.g., Kupffer cells, e.g., does not reduce the number of tissue-resident

macrophages, e.g., Kupffer cells, by more than 4, 6, 8, 10, or 15%, in vivo, e.g., as measured using an immunohistochemistry analysis, e.g., as measured using methods described in Example 8 with respect to FIGs. 7B and 7D;

(x) the multispecific molecule does not inhibit or does not substantially inhibit CSF-1 dependent cell survival of CSF1R-positive, CCR2-negative cells, e.g., does not reduce CSF-1 dependent cell survival of CSF1R-positive, CCR2-negative cells by more than 5, 10, or 15%, e.g., as measured using a cell viability MTT assay, e.g., as measured using methods described in Example 9 with respect to FIG. 8A;

(xi) the multispecific molecule increases CD8+ T cell tumor infiltration in vivo, e.g., increases % CD8+ T cells in CD3+ T cells in tumor by at least 1.5, 2, or 2.5-fold, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 10 with respect to FIG. 9;

(xii) the multispecific molecule reduces Treg frequency in tumor in vivo, e.g., reduces Treg frequency in tumor by at least 15, 20, 25, or 30%, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 11 with respect to FIG. 10A;

(xiii) the multispecific molecule increases the CD8+ T cell/Treg ratio in tumor in vivo, e.g., increases the CD8+ T cell/Treg ratio in tumor by at least 2.5, 3, 3.5, 4, or 4.5-fold, e.g., as measured using a flow cytometry analysis, e.g., as measured using methods described in Example 11 with respect to FIG. 10B; or

(xiv) the multispecific molecule reduces tumor growth and/or increases survival of a tumor-bearing animal, e.g., as measured using methods described in Example 12 with respect to FIGs. 11A and 11B.

52. The multispecific molecule of claim 50 or 51, wherein the anti-CSF1R binding moiety and the anti-CCR2 binding moiety are, independently, a full antibody (e.g., an antibody that includes at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains), or an antigen-binding fragment (e.g., a Fab, F(ab')₂, Fv, a scFv, a single domain antibody, or a diabody (dAb)).

53. The multispecific molecule of any one of claims 50-52, wherein the anti-CSF1R binding moiety and/or the anti-CCR2 binding moiety comprises a heavy chain constant region chosen from IgG1, IgG2, IgG3, or IgG4, or a fragment thereof.

54. The multispecific molecule of any one of claims 50-52, wherein the anti-CSF1R binding moiety and/or the anti-CCR2 binding moiety comprises a heavy chain constant region that can mediate antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).

55. The multispecific molecule of any one of claims 50-52, wherein the anti-CSF1R binding moiety comprises a first heavy chain constant region (e.g., a first Fc region) and the anti-CCR2 binding moiety comprises a second heavy chain constant region (e.g., a second Fc region), wherein the first heavy chain constant region comprises one or more mutations that increase heterodimerization of the first heavy chain constant region and the second heavy chain constant region, relative to a naturally-existing heavy chain constant region, and/or wherein the second heavy chain constant region comprises one or more mutations that increase heterodimerization of the second heavy chain constant region and the first heavy chain constant region, relative to a naturally-existing heavy chain constant region.

56. The multispecific molecule of any one of claims 50-55, wherein the anti-CSF1R binding moiety and/or the anti-CCR2 binding moiety comprises a light chain constant region chosen from the light chain constant regions of kappa or lambda, or a fragment thereof.

57. The multispecific molecule of any one of claims 50-55, wherein the anti-CSF1R binding moiety comprises a kappa light chain constant region, or a fragment thereof, and the anti-CCR2 binding moiety comprises a lambda light chain constant region, or a fragment thereof.

58. The multispecific molecule of any one of claims 50-55, wherein the anti-CSF1R binding moiety comprises a lambda light chain constant region, or a fragment thereof, and the anti-CCR2 binding moiety comprises a kappa light chain constant region, or a fragment thereof.

59. The multispecific molecule of any one of claims 50-55, wherein the anti-CSF1R binding moiety and the anti-CCR2 binding moiety have a common light chain variable region.

60. The multispecific molecule of any one of claims 50-59, further comprising a heavy chain constant region (e.g., an Fc region) chosen from the heavy chain constant regions of IgG1, IgG2, and IgG4, more particularly, the heavy chain constant region of human IgG1, IgG2 or IgG4.

61. The multispecific molecule of any one of claims 50-59, further comprising a heavy chain constant region that can mediate antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).

62. The multispecific molecule of any one of claims 50-61, comprising an anti-CSF1R antibody molecule and an anti-CCR2 antibody molecule, wherein:

(i) the anti-CSF1R antibody molecule comprises a first polypeptide and a second polypeptide, wherein the first polypeptide comprises a first light chain variable region (VL) and a first light chain constant region (CL), and the second polypeptide comprises a first heavy chain variable region (VH), a first heavy chain constant region 1 (CH1), and optionally, a first CH2 and a first CH3, and

(ii) the anti-CCR2 antibody molecule comprises a third polypeptide and a fourth polypeptide, wherein the third polypeptide comprises a second VL and a second CL, and the fourth polypeptide comprises a second VH, a second CH1, and optionally, a second CH2 and a second CH3.

63. The multispecific molecule of any one of claims 50-62, wherein:

(i) the anti-CSF1R antibody molecule binds to CSF1R monovalently, and/or

(ii) the anti-CCR2 antibody molecule binds to CCR2 monovalently.

64. The multispecific molecule of any one of claims 50-63, wherein the multispecific molecule binds to CSF1R monovalently, and/or binds to CCR2 monovalently.

65. The multispecific molecule of any one of claims 50-63, wherein the multispecific molecule binds to CSF1R monovalently, and binds to CCR2 monovalently.

66. The multispecific molecule of any one of claims 50-65, wherein:

(i) the multispecific molecule inhibits CSF1R in the presence of CCR2, optionally wherein the multispecific molecule reduces an activity of CSF1R (e.g., CSF1R signaling, e.g., CSF1-induced CSF1R signaling) in a cell, e.g., by at least 40, 50, 60, 70, 80, or 90%, when the cell expresses both CSF1R and CCR2 on the cell surface, and/or

(ii) the multispecific molecule does not inhibit or does not substantially inhibit CSF1R in the absence of CCR2, optionally wherein the multispecific molecule does not reduce an activity of CSF1R (e.g., CSF1R signaling, e.g., CSF1-induced CSF1R signaling), or does not reduce an activity of CSF1R by more than 2, 4, 6, 8, 10, or 15%, when the cell expresses CSF1R but not CCR2 on the cell surface.

67. The multispecific molecule of any one of claims 50-66, wherein:

(i) the multispecific molecule inhibits CCR2 in the presence of CSF1R, optionally wherein the multispecific molecule reduces an activity of CCR2 in a cell, e.g., by at least 40, 50, 60, 70, 80, or 90%, when the cell expresses both CCR2 and CSF1R on the cell surface, and/or

(ii) the multispecific molecule does not inhibit or does not substantially inhibit CCR2 in the absence of CSF1R, optionally wherein the multispecific molecule does not reduce an activity of CCR2, or does not reduce an activity of CCR2 by more than 2, 4, 6, 8, 10, or 15%, when the cell expresses CCR2 but not CSF1R on the cell surface.

68. An isolated multispecific, e.g., a bispecific, molecule, comprising:

(i) a first binding moiety that binds to a molecule that mediates the trafficking of monocytes, e.g., inflammatory monocytes, e.g., Ly6C^{Hi} CCR2^+ $\text{CX3CR1}^{\text{Lo}}$ inflammatory monocytes, optionally wherein the first binding moiety binds to CCR2; and

(ii) a second binding moiety that binds to a molecule that mediates the maturation and/or survival of monocytes and/or macrophages at an inflamed tissue, optionally wherein the second binding moiety binds to CSF1R.

69. An isolated multispecific, e.g., a bispecific, molecule, comprising:

- (i) a first binding moiety that reduces recruitment of inflammatory monocytes to tumor, optionally wherein the first binding moiety binds to and/or inhibits CCR2; and
- (ii) a second binding moiety that reduces maturation and/or survival of monocytes and/or macrophages in the tumor microenvironment, optionally wherein the second binding moiety binds to and/or inhibits CSF1R.

70. The multispecific molecule of any one of claims 50-69, comprising one or more additional binding moieties (e.g., a third binding moiety, a fourth binding moiety, (e.g., a trispecific or a tetraspecific molecule), optionally wherein the third binding moiety is a third IMC binding moiety or a tumor targeting moiety.

71. An isolated nucleic acid molecule encoding the multispecific molecule (e.g., antibody) of any one of claims 1-70.

72. A vector, e.g., an expression vector, comprising the nucleic acid molecule of claim 71.

73. A cell, e.g., a host cell, comprising the nucleic acid molecule of claim 71 or the vector of claim 72.

74. A method of making, e.g., producing, the multispecific molecule of any one of claims 1-70, comprising culturing the cell, e.g., host cell, of claim 73, under suitable conditions, e.g., conditions suitable for gene expression and/or heterodimerization.

75. A pharmaceutical composition comprising the multispecific molecule of any one of claims 1-70 and a pharmaceutically acceptable carrier, excipient, or stabilizer.

76. A method of treating a cancer in a subject, comprising administering to the subject in need thereof the multispecific molecule of any one of claims 1-70, wherein the multispecific molecule is administered in an amount effective to treat the cancer.

77. A method of treating a cancer in a subject, comprising administering to the subject in need thereof the multispecific molecule of any one of claims 1-70, wherein the multispecific molecule is administered in an amount effective to reduce the number of TAMs (e.g., the number of TAMs in or near a tumor in the subject), inhibit the proliferation of TAMs (e.g., the proliferation of TAMs in or near a tumor in the subject), or reduce or inhibit macrophage infiltration into a tumor in the subject.

78. A method of treating a cancer in a subject by reducing a portion of a population of TAMs, comprising administering to the subject in need thereof the multispecific molecule of any one of claims 1-70, wherein the multispecific molecule is administered in an amount effective to inhibit or deplete a portion of the population of TAMs.

79. A method of reducing the proliferation of a portion of a population of TAMs in a subject (e.g., in a subject having cancer, e.g., a solid tumor), comprising administering to the subject in need thereof the multispecific molecule of any one of claims 1-70, wherein the multispecific molecule is administered in an amount effective to reduce the proliferation of a portion of the population of TAMs.

80. A method of inhibiting or depleting a portion of a population of TAMs in a subject having a cancer (e.g., a tumor), comprising administering to the subject the multispecific molecule of any one of claims 1-70, wherein the multispecific molecule is administered in an amount effective to reduce the number of tumor infiltrating macrophages, inhibit the proliferation of tumor infiltrating macrophages, or reduce macrophage infiltration into a tumor.

81. The method of any one of claims 76-80, wherein the cancer is a solid tumor cancer or a metastatic lesion.

82. The method of claim 81, wherein the solid tumor cancer is one or more of pancreatic cancer (e.g., pancreatic adenocarcinoma), breast cancer, colorectal cancer, lung cancer (e.g.,

small or non-small cell lung cancer), skin cancer (e.g., melanoma), ovarian cancer, liver cancer, or brain cancer (e.g., glioma).

83. The method of claim 81, wherein the cancer is characterized as containing TAMs, is associated with the presence of TAMs, TAMs are in and/or form part of the cancer (e.g., tumor), or TAMs have been detected in or near the solid tumor.

84. The method of any one of claims 76-83, comprising identifying the presence of TAMs in or near the cancer (e.g., tumor) in the subject.

85. The method of any one of claims 77-84, wherein the TAMs express CXCR2 and CCR2, CCR2 and CSF1R, CSF1R and CXCR2, or CCR2, CXCR2, and CSF1R.

86. The method of any one of claims 76-85, further comprising administering a second therapeutic treatment.

87. The method of claim 86, wherein the second therapeutic treatment comprises a therapeutic agent (e.g., a chemotherapeutic agent, a biologic agent, hormonal therapy), radiation, or surgery.

88. The method of claim 87, wherein the therapeutic agent is chosen from: a chemotherapeutic agent, or a biologic agent.

89. The method of claim 87, wherein the therapeutic agent is a checkpoint inhibitor.

90. The method of claim 89, wherein the check point inhibitor is selected from the group consisting of an anti-CTLA4 antibody, an anti-PD1 antibody (e.g., Nivolumab, Pembrolizumab or Pidilizumab), an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-CD160 antibody, an anti-2B4 antibody, an anti-CD80 antibody, an anti-CD86 antibody, an anti-B7-H3 (CD276) antibody, an anti-B7-H4 (VTCN1) antibody, an anti-HVEM (TNFRSF14 or CD270) antibody, an anti-BTLA antibody, an anti-KIR antibody, an

anti-MHC class I antibody, an anti-MHC class II antibody, an anti-GAL9 antibody, an anti-VISTA antibody, an anti-BTLA antibody, an anti-TIGIT antibody, an anti-LAIR1 antibody, and an anti-A2aR antibody.

91. A method of treating a cancer in a subject, comprising administering to the subject in need thereof the multispecific molecule of any one of claims 1-70, wherein the multispecific molecule is administered in an amount effective to reduce the number of MDSCs (e.g., the number of MDSCs in or near a tumor in the subject), inhibit the proliferation of MDSCs (e.g., the number of MDSCs in or near a tumor in the subject), or reduce or inhibit MDSC infiltration into a tumor in the subject.

92. A method of treating a cancer in a subject by reducing a portion of a population of MDSCs, comprising administering to the subject in need thereof the multispecific molecule of any one of claims 1-70, wherein the multispecific molecule is administered in an amount effective to inhibit or deplete a portion of the population of MDSCs.

93. A method of reducing the proliferation of a portion of a population of MDSCs in a subject (e.g., in a subject having cancer, e.g., a solid tumor), comprising administering to the subject in need thereof the multispecific molecule of any one of claims 1-70, wherein the multispecific molecule is administered in an amount effective to reduce the proliferation of a portion of the population of MDSCs.

94. A method of inhibiting or depleting a portion of a population of MDSCs in a subject having a cancer (e.g., a tumor), comprising administering to the subject the multispecific molecule of any one of claims 1-70, wherein the multispecific molecule is administered in an amount effective to reduce the number of MDSCs, inhibit the proliferation of MDSCs, or reduce MDSC infiltration into a tumor.

95. The method of any one of claims 91-94, wherein the cancer is a solid tumor cancer or a metastatic lesion.

96. The method of claim 95, wherein the solid tumor cancer is one or more of pancreatic cancer (e.g., pancreatic adenocarcinoma), breast cancer, colorectal cancer, lung cancer (e.g., small or non-small cell lung cancer), skin cancer (e.g., melanoma), ovarian cancer, liver cancer, or brain cancer (e.g., glioma).

97. The method of claim 95, wherein the cancer is characterized as containing MDSCs, is associated with the presence of MDSCs, MDSCs are in and/or form part of the cancer (e.g., tumor), or MDSCs have been detected in or near the solid tumor.

98. The method of any one of claims 91-97, comprising identifying the presence of MDSCs in or near the cancer (e.g., tumor) in the subject.

99. The method of any one of claims 91-98, further comprising administering a second therapeutic treatment.

100. The method of claim 99, wherein the second therapeutic treatment comprises a therapeutic agent (e.g., a chemotherapeutic agent, a biologic agent, hormonal therapy), radiation, or surgery.

101. The method of claim 100, wherein the therapeutic agent is selected from: a chemotherapeutic agent, or a biologic agent.

102. The method of claim 100, wherein the therapeutic agent is a checkpoint inhibitor.

103. The method of claim 102, wherein the check point inhibitor is selected from the group consisting of an anti-CTLA4 antibody, an anti-PD1 antibody (e.g., Nivolumab, Pembrolizumab or Pidilizumab), an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-CD160 antibody, an anti-2B4 antibody, an anti-CD80 antibody, an anti-CD86 antibody, an anti-B7-H3 (CD276) antibody, an anti-B7-H4 (VTCN1) antibody, an anti-HVEM (TNFRSF14 or CD270) antibody, an anti-BTLA antibody, an anti-KIR antibody, an anti-MHC class I antibody, an anti-MHC class II antibody, an anti-GAL9 antibody,

an anti-VISTA antibody, an anti-BTLA antibody, an anti-TIGIT antibody, an anti-LAIR1 antibody, and an anti-A2aR antibody.

104. A method of treating a fibrotic disease or disorder in a subject in need thereof, comprising administering to the subject an effective amount of the multispecific molecule of any one of claims 1-70, thereby treating the fibrotic disease or disorder.

105. The method of claim 104, wherein the fibrotic disease or disorder is a fibrotic disease or disorder of the lung, the liver, the heart or vasculature, the kidney, the pancreas, the skin, the gastrointestinal tract, the bone marrow or a hematopoietic tissue, the nervous system, the eye, or a combination thereof.

106. The method of claim 104, wherein the fibrotic disease or disorder is lung fibrosis (e.g., Idiopathic pulmonary fibrosis (IPF)) or liver fibrosis (e.g., Nonalcoholic steatohepatitis (NASH)).

FIG. 1

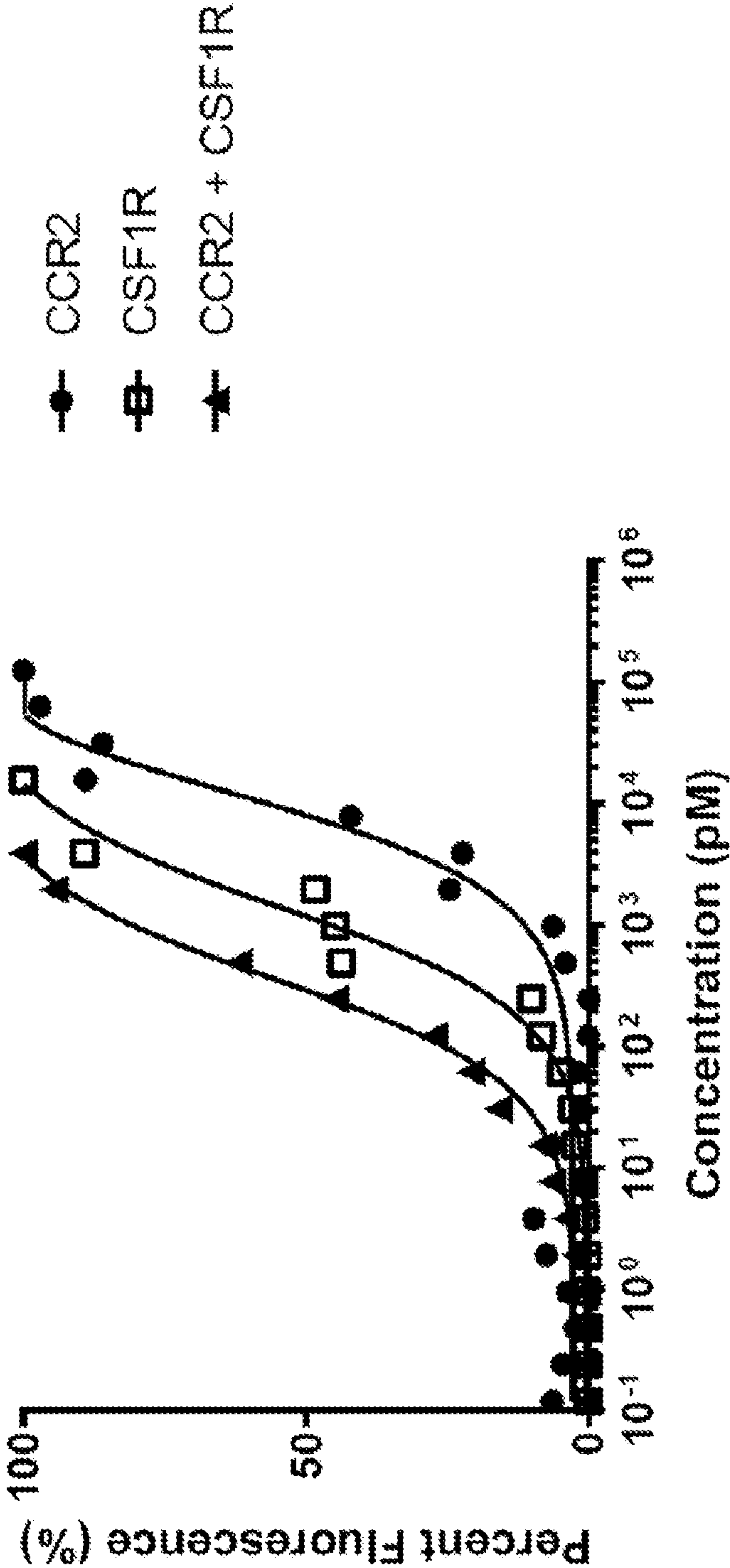


FIG. 2

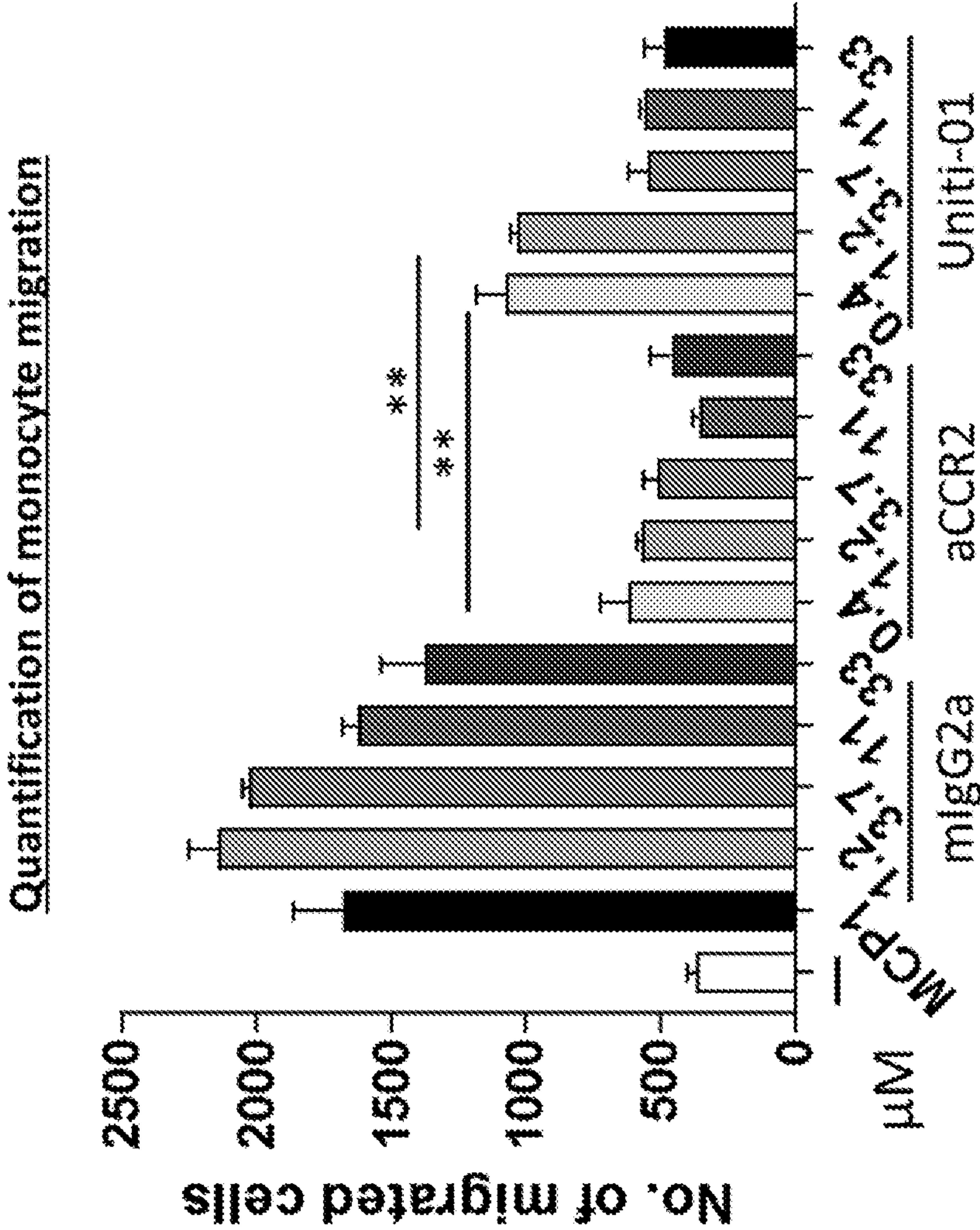


FIG. 3A

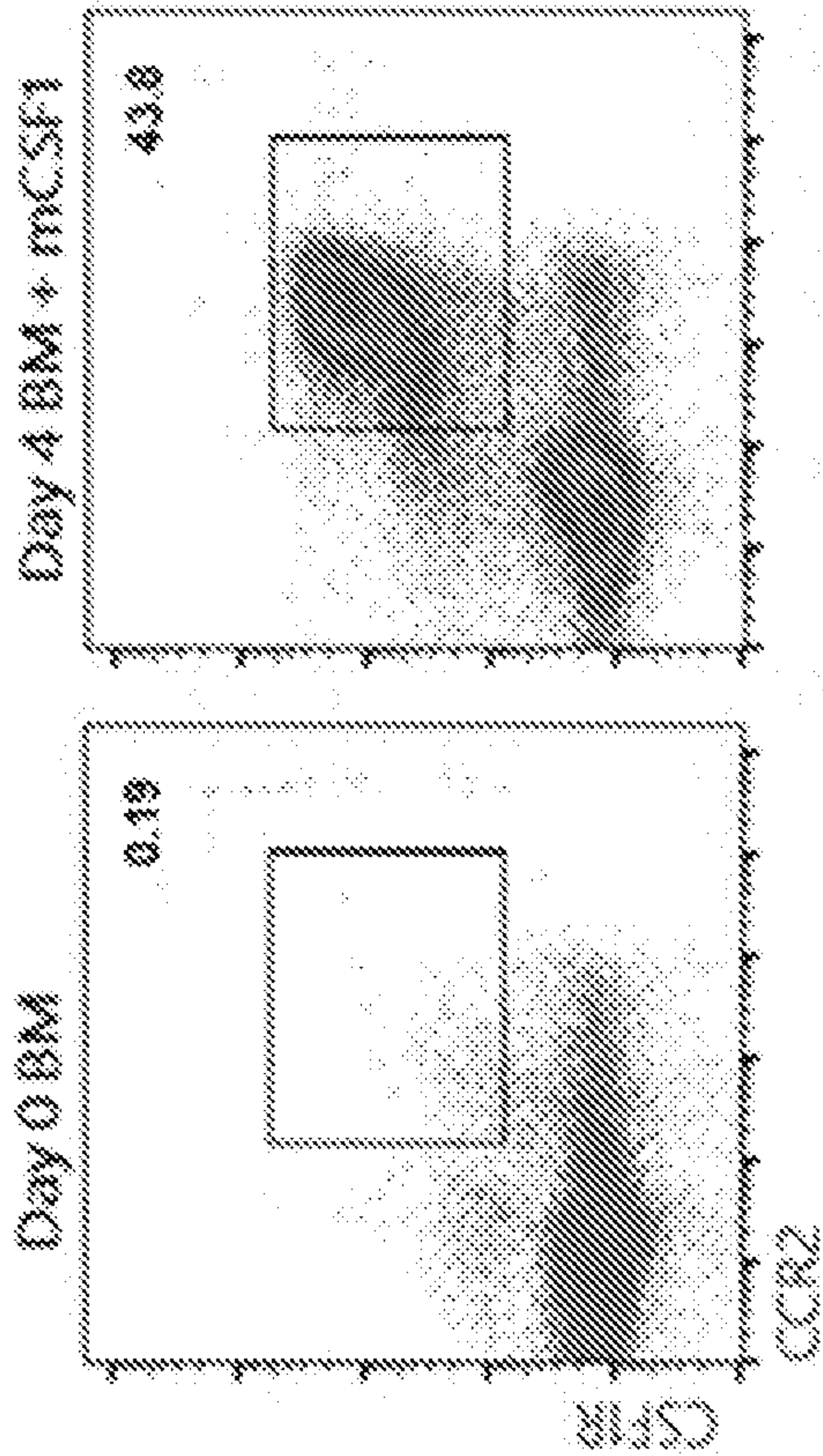


FIG. 3B

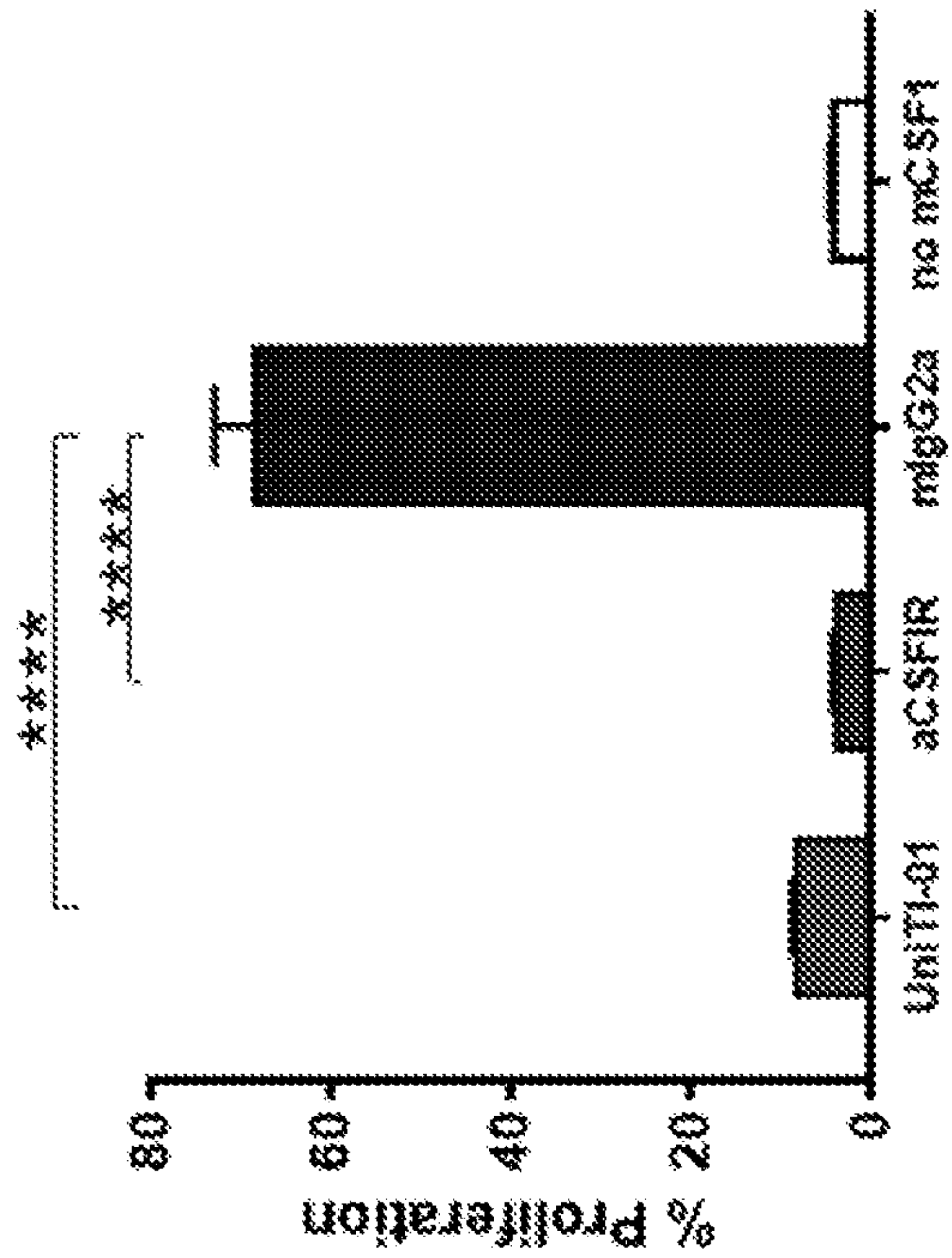


FIG. 4

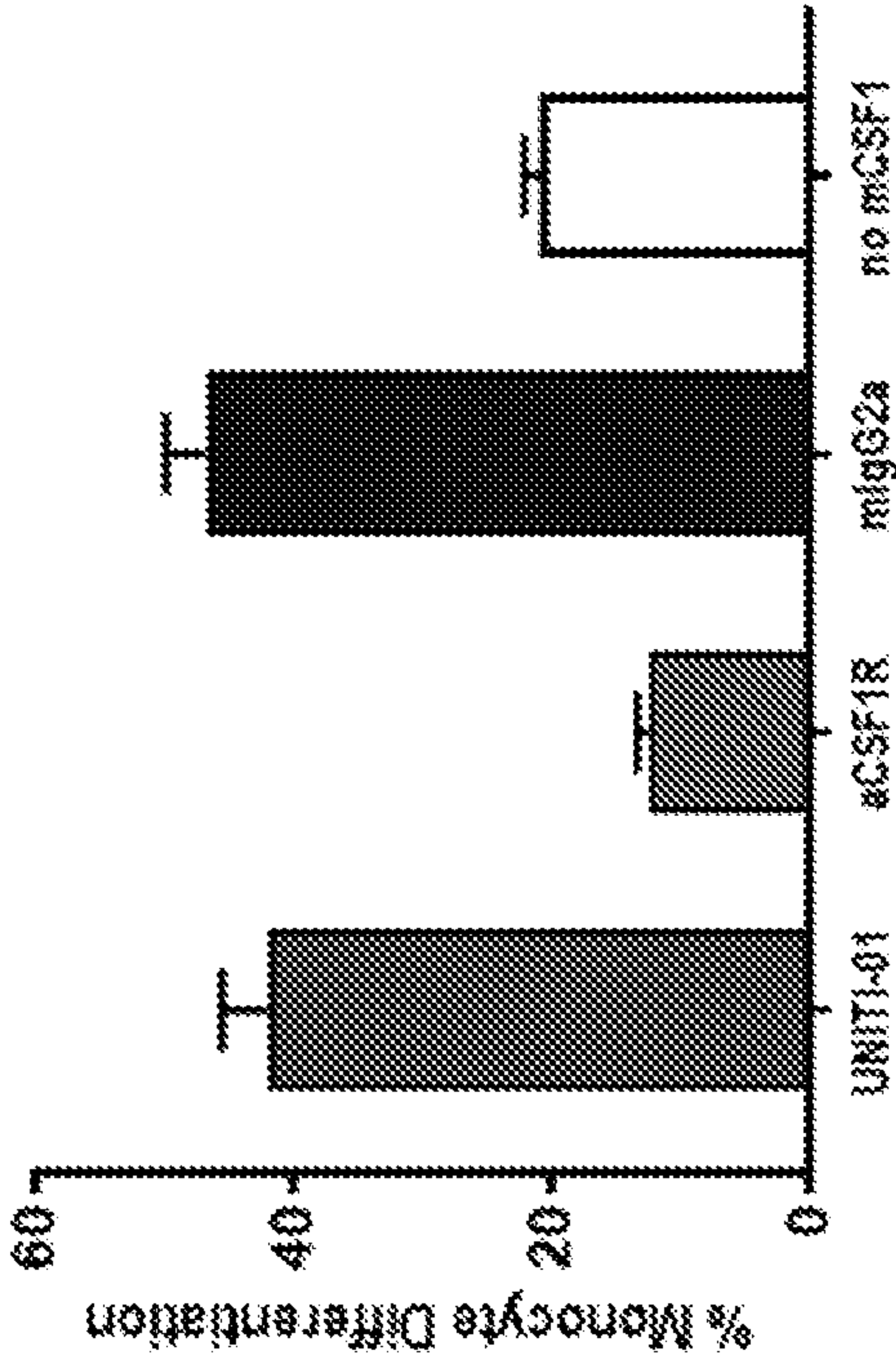


FIG. 5

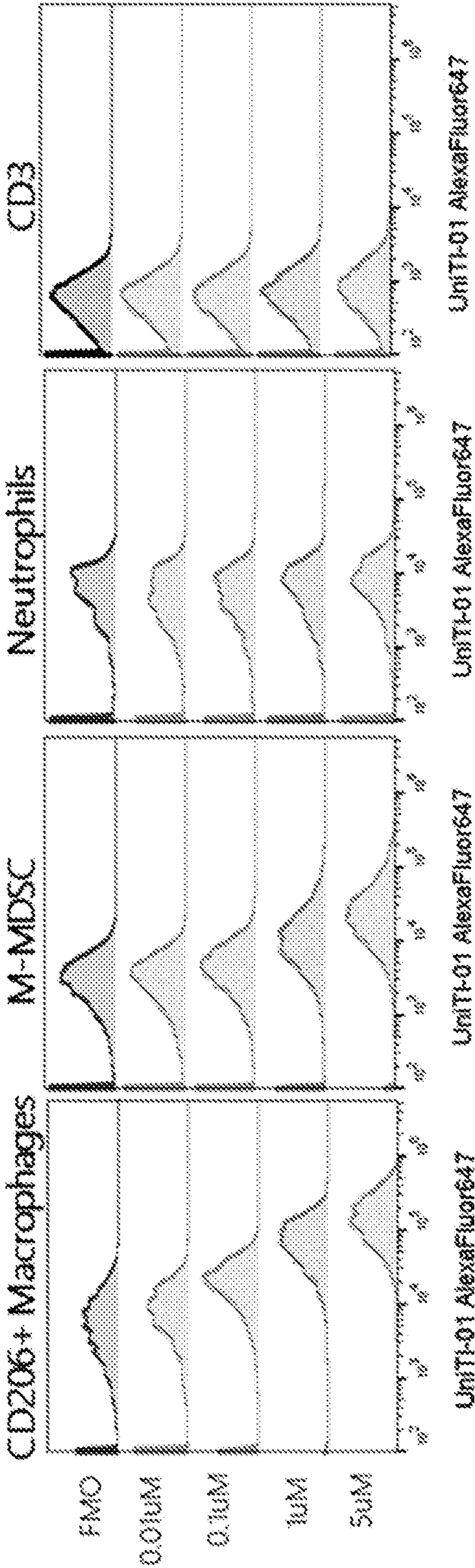


FIG. 6

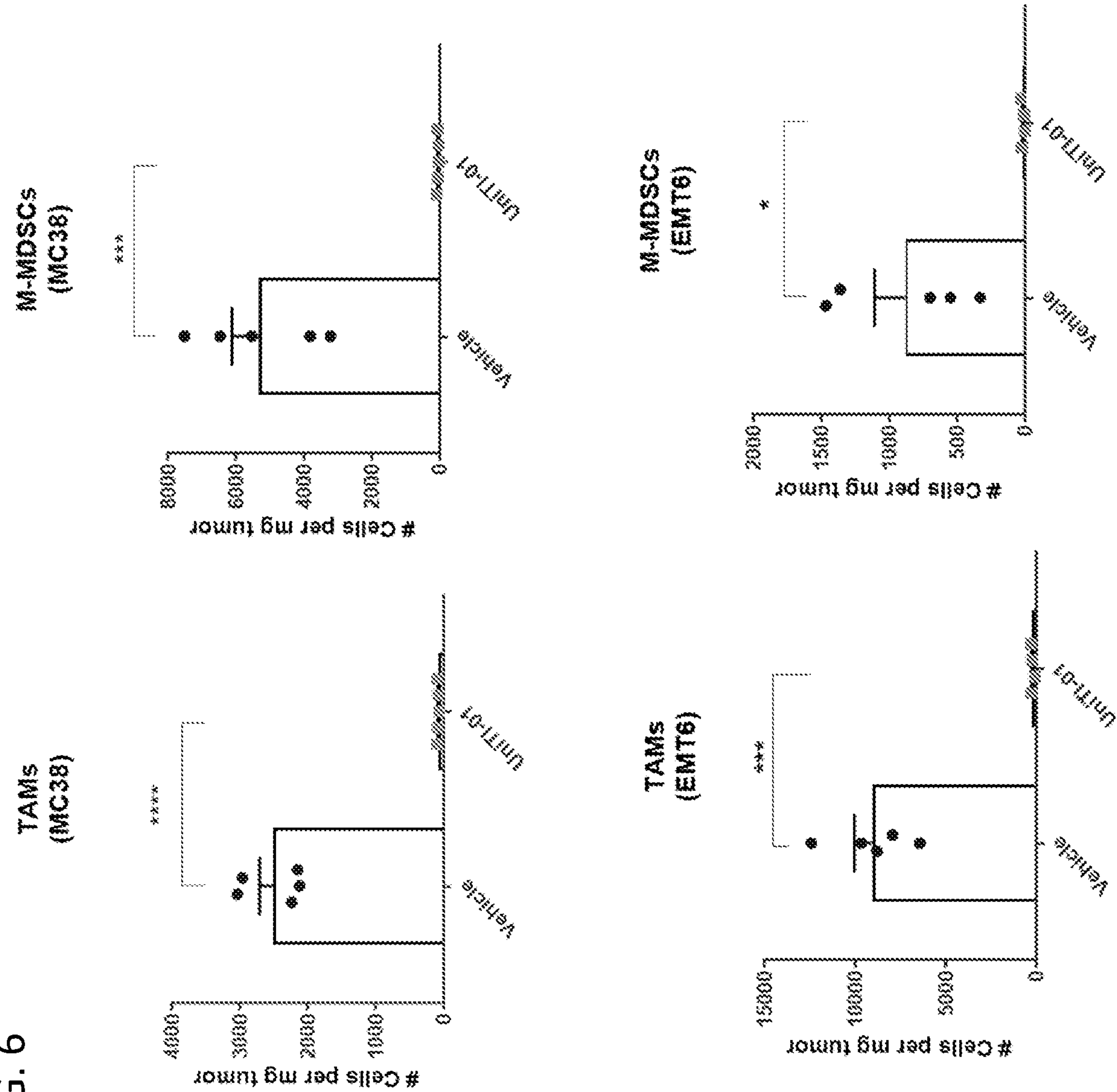


FIG. 7A

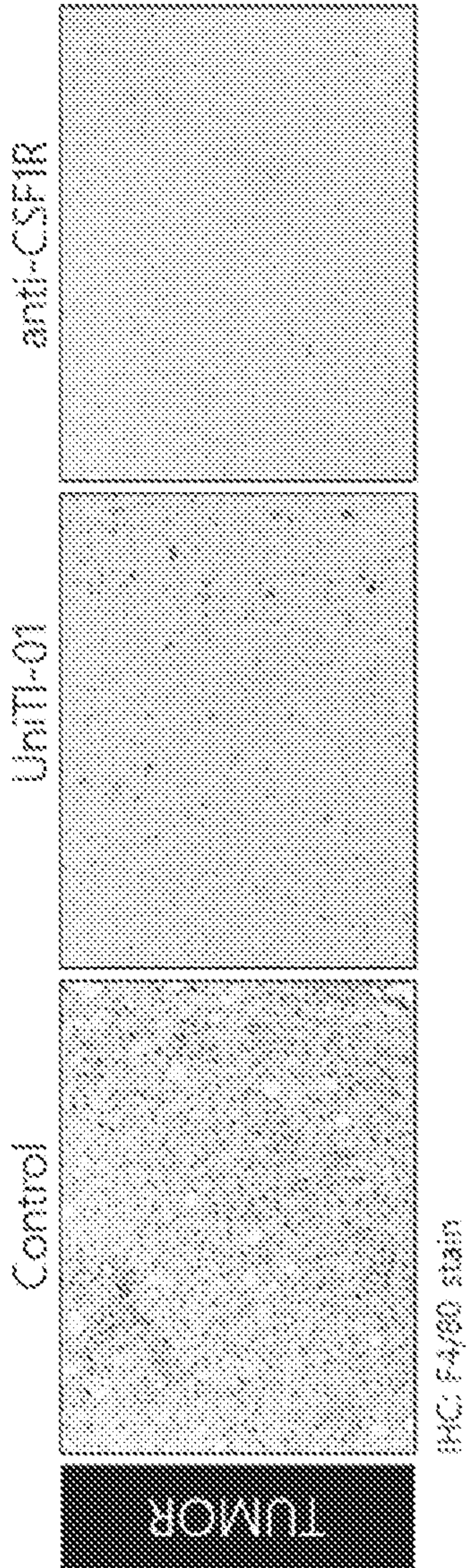


FIG. 7C

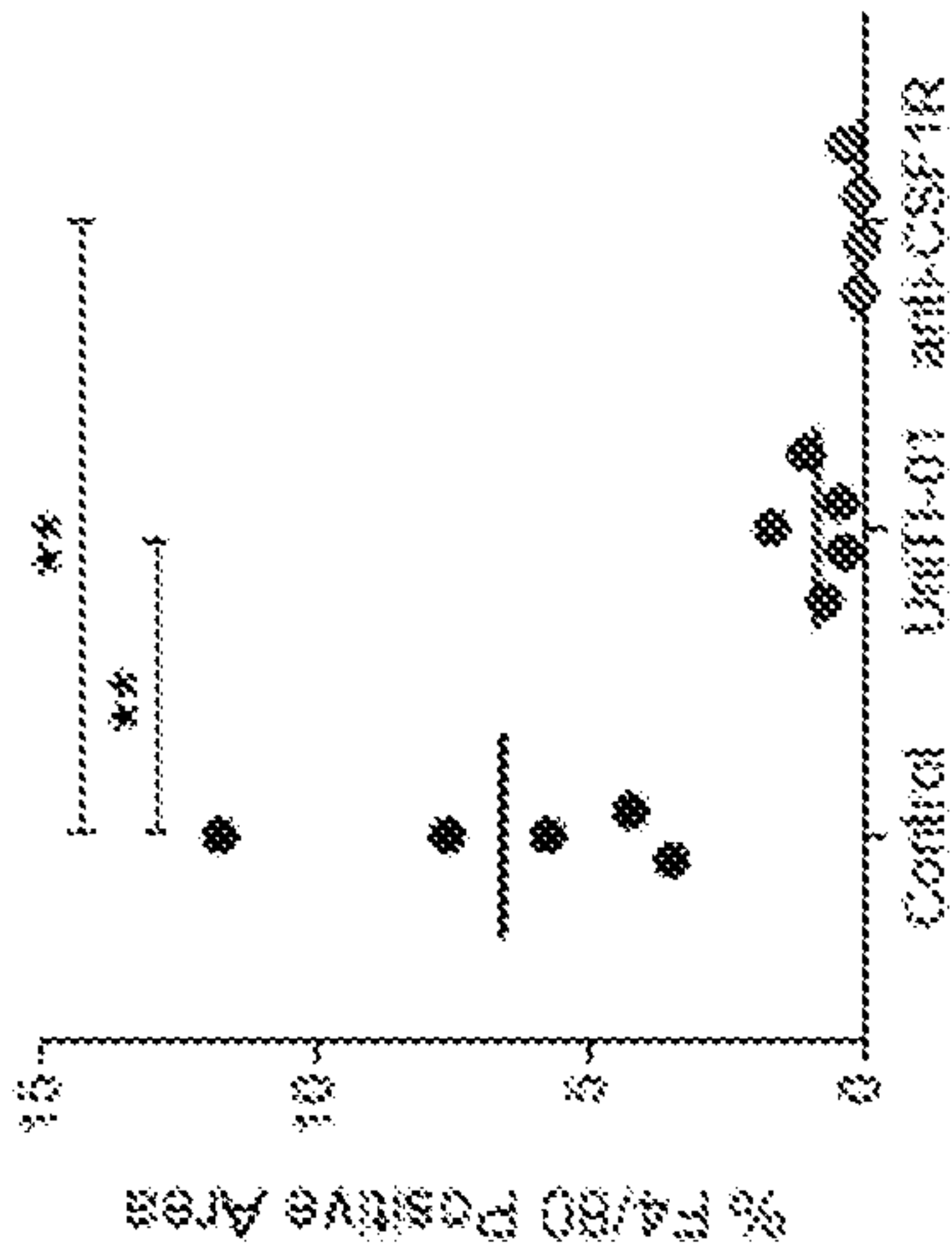


FIG. 7B

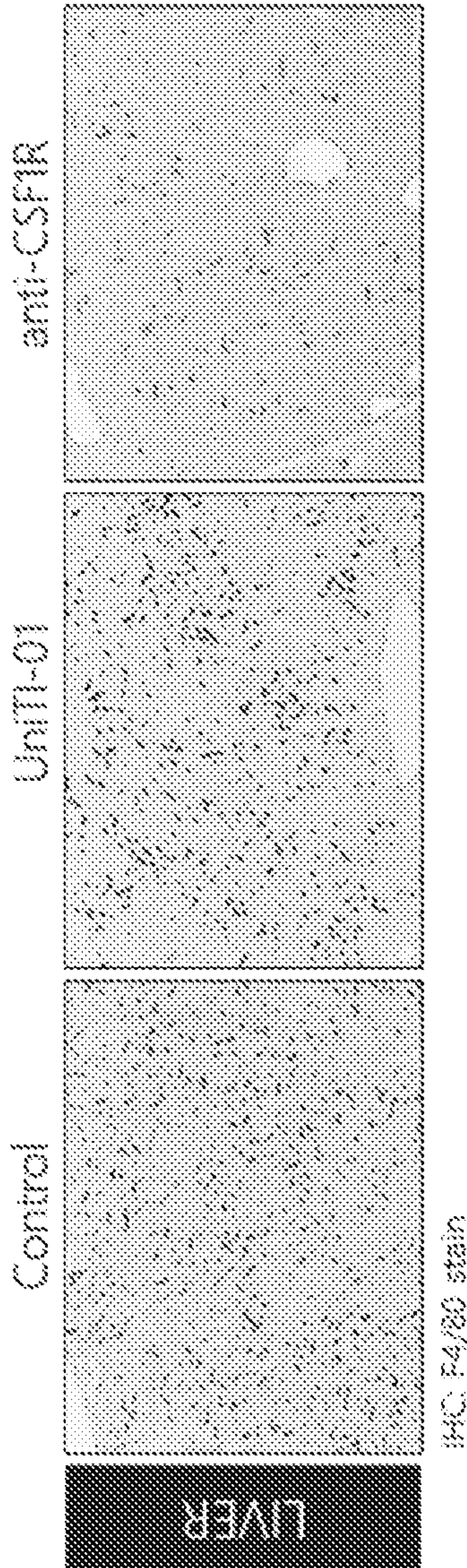
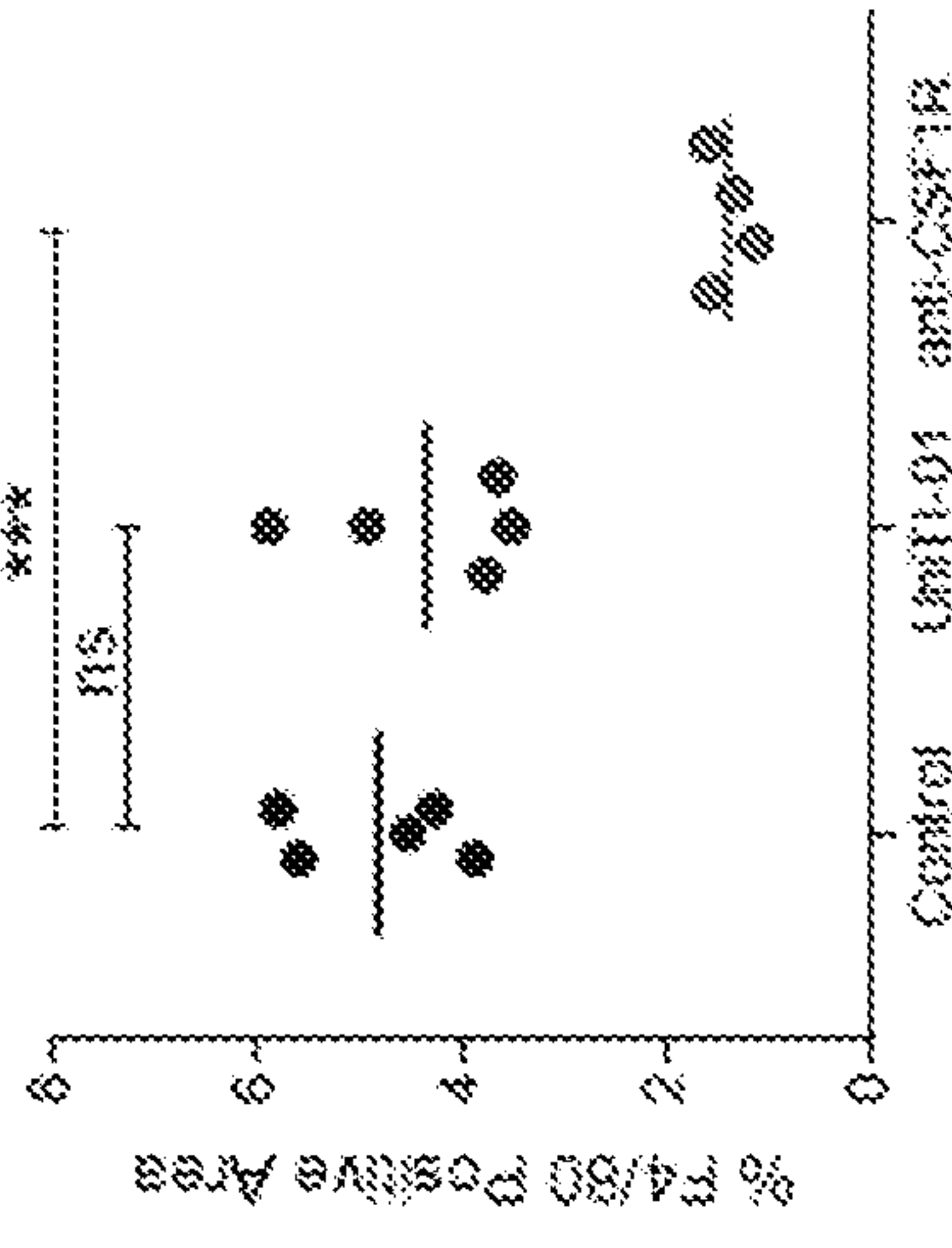


FIG. 7D



p < 0.01, *p < 0.001; One-way ANOVA

FIG. 8A

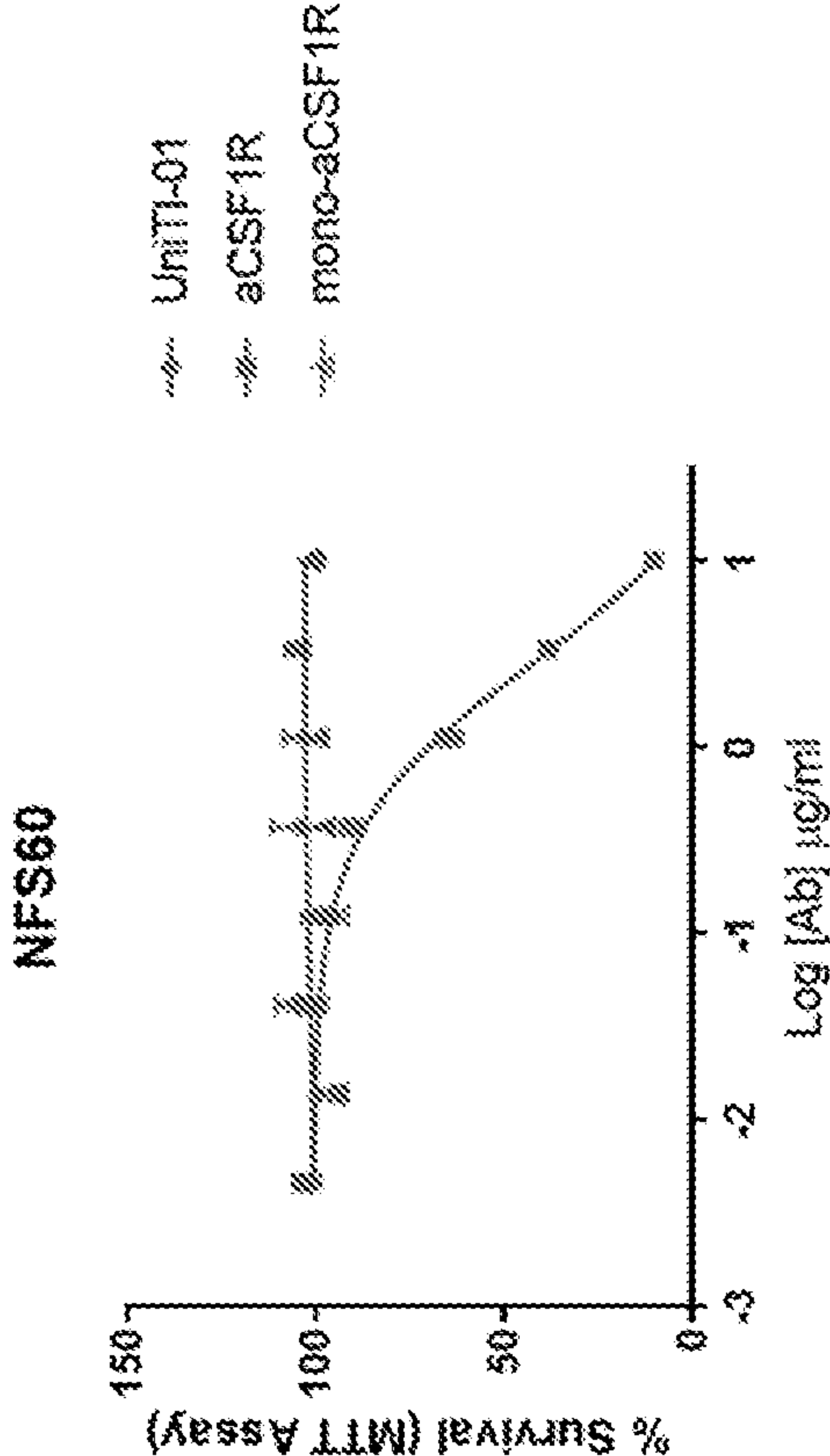


FIG. 8B

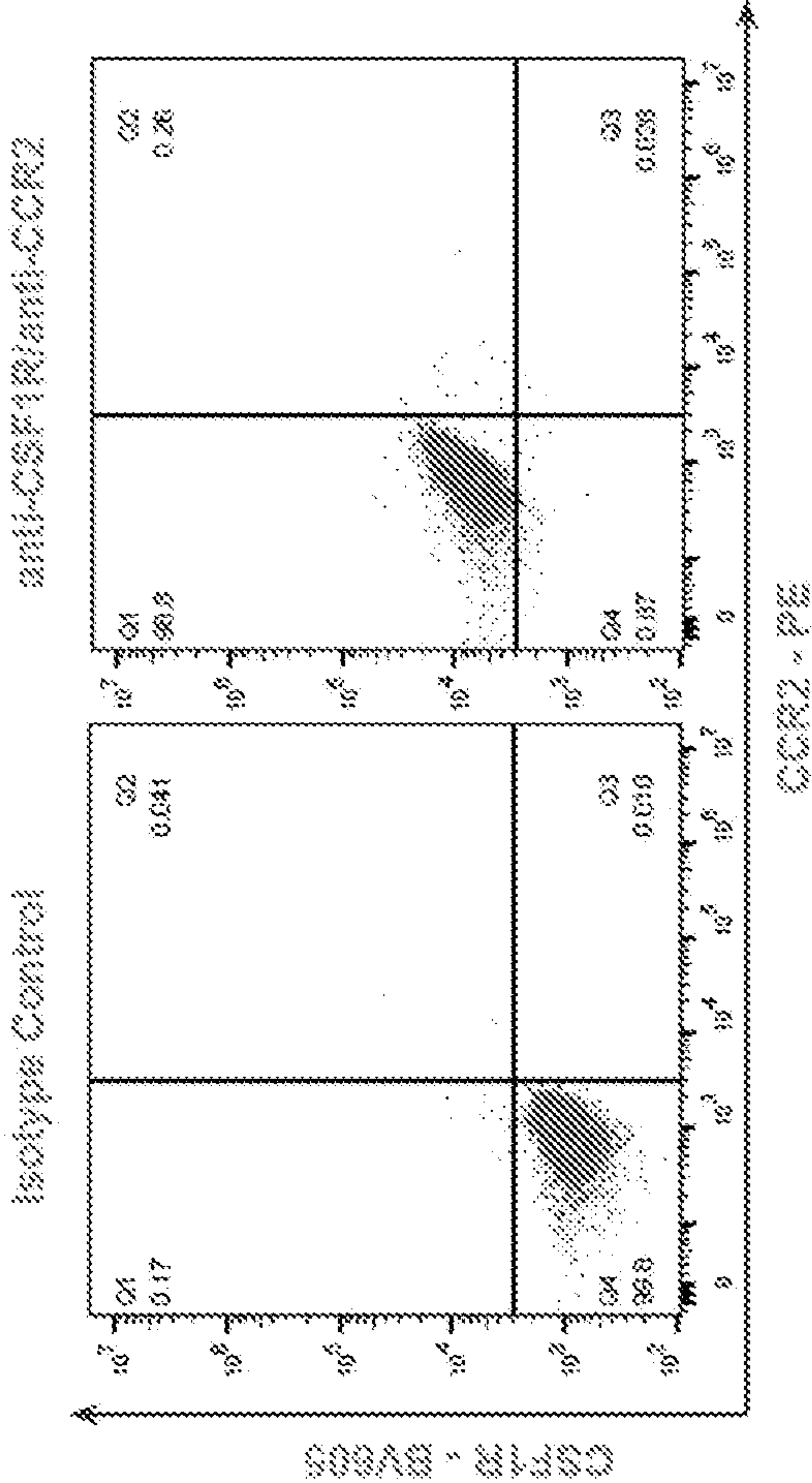


FIG. 9

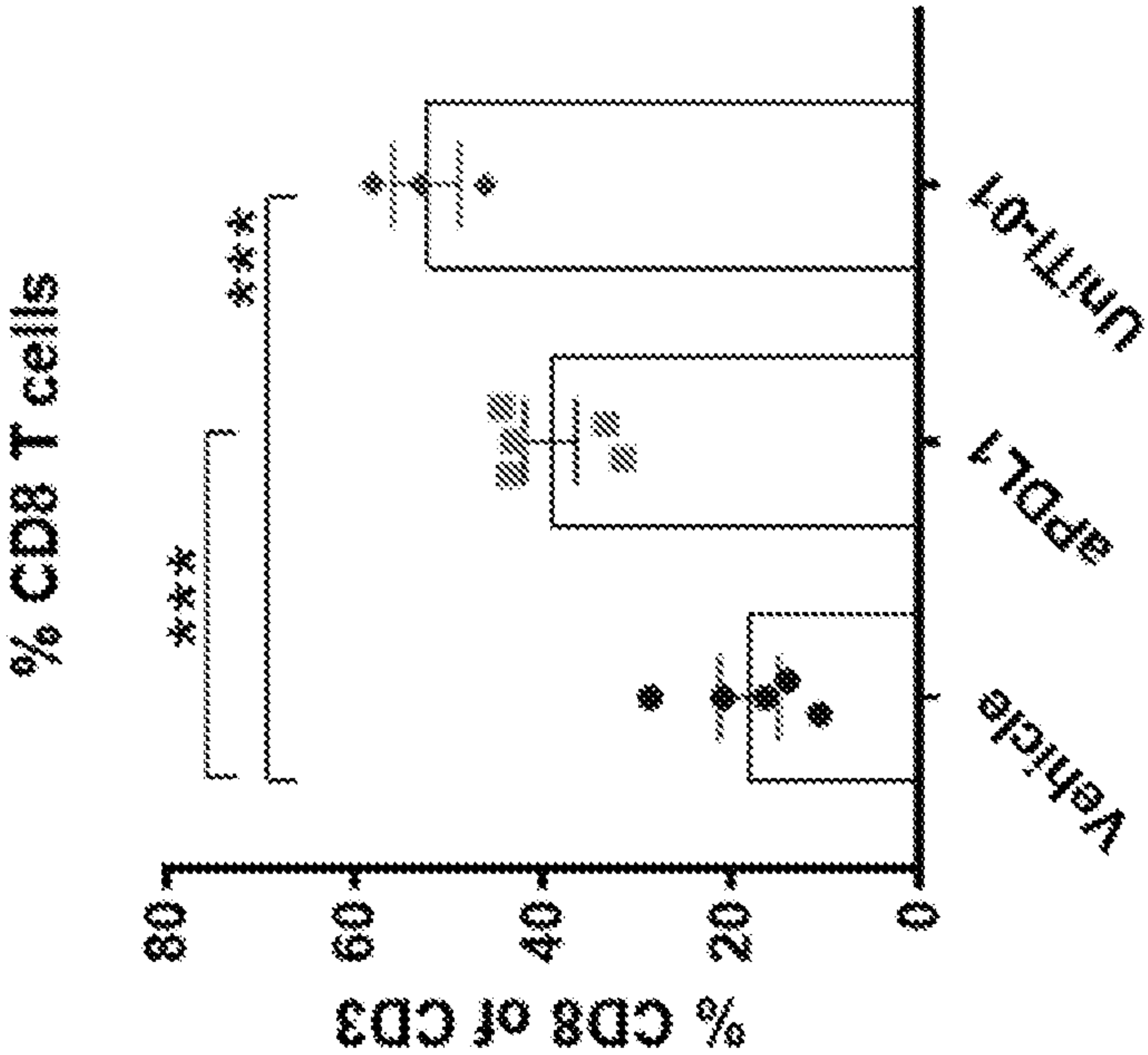


FIG. 10A

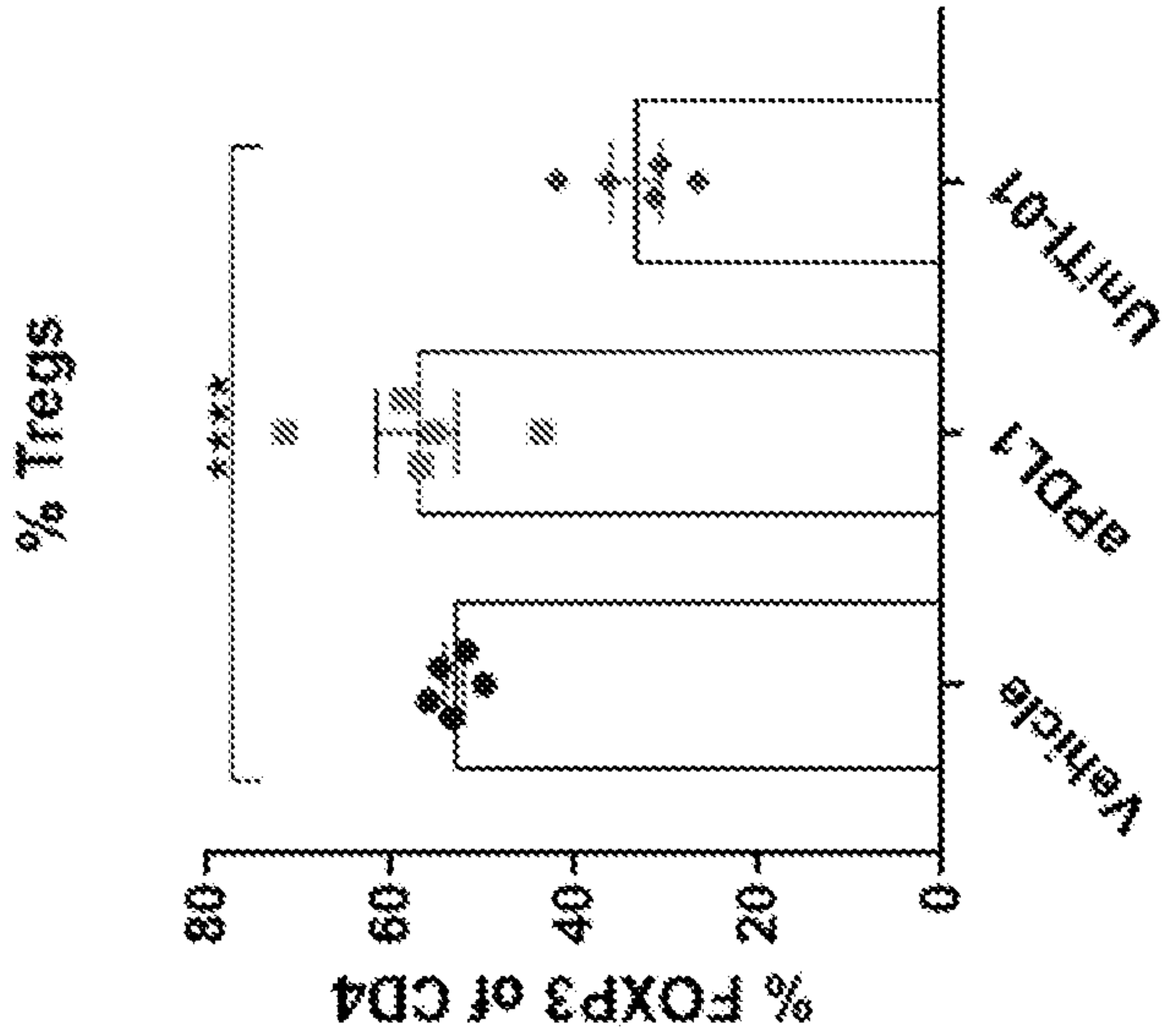


FIG. 10B

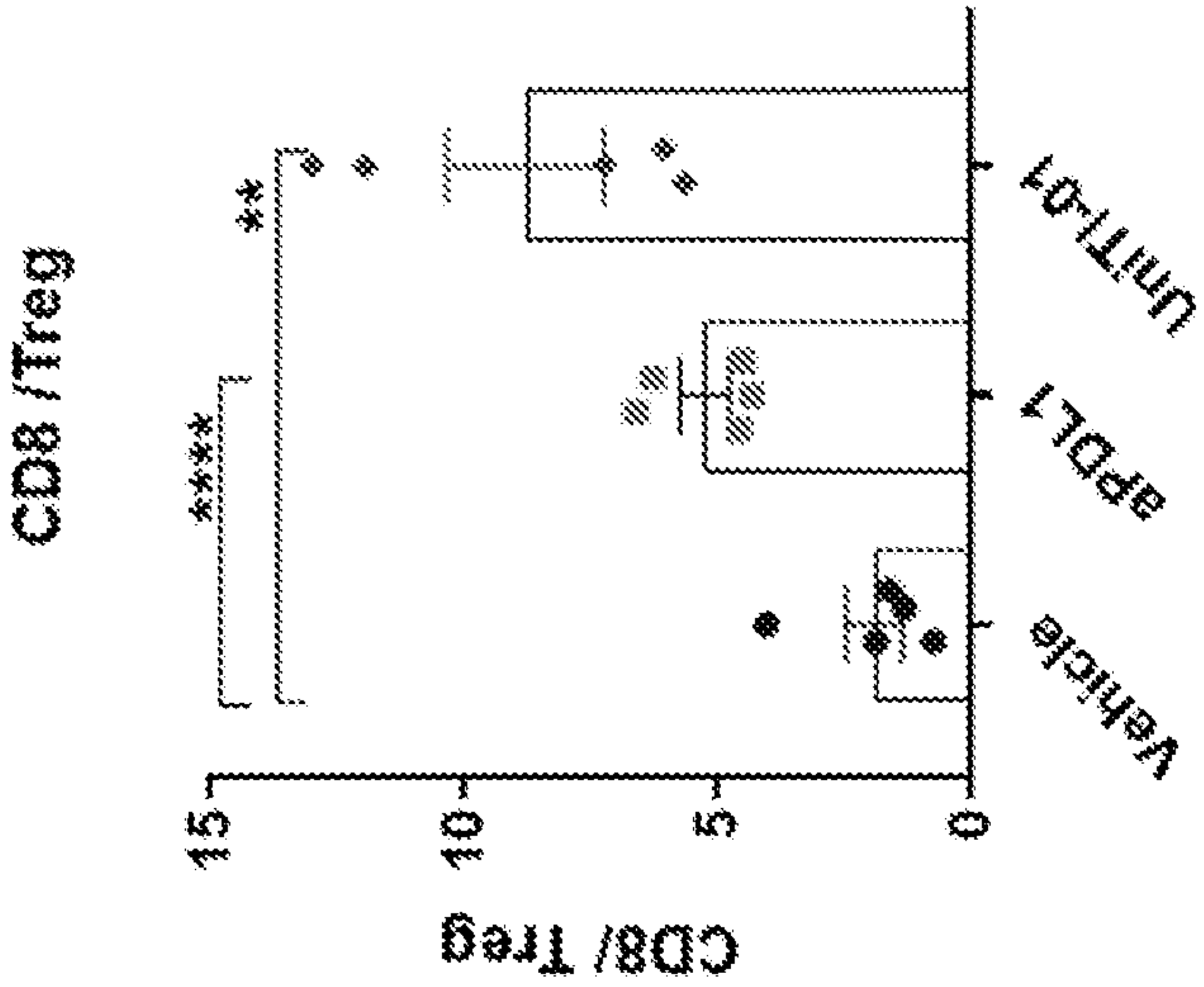


FIG. 11A

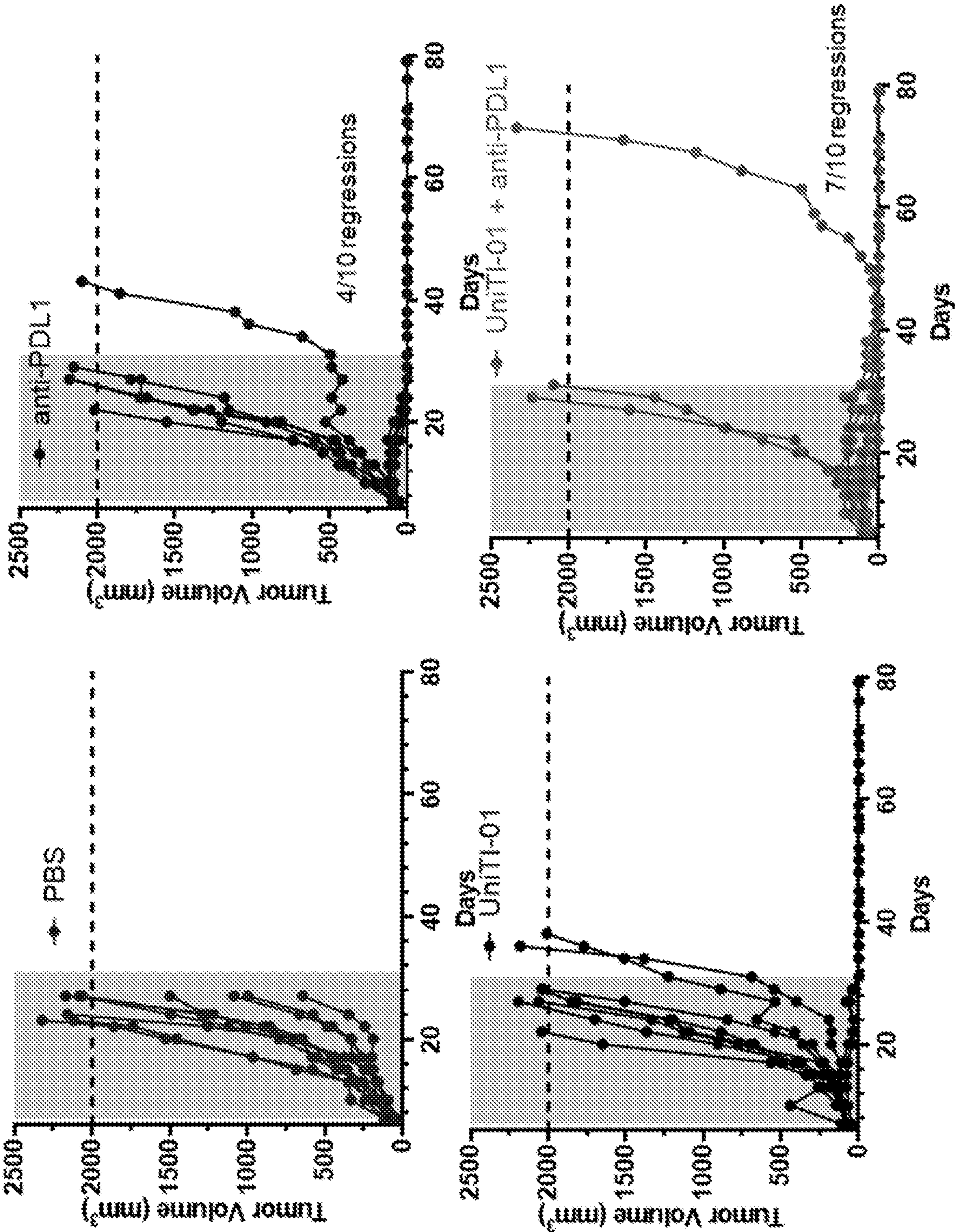


FIG. 11B

