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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2023 (14.12.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/240272 A2

(51) International Patent Classification:
C07K 16/42 (2006.01) *A61K 39/395* (2006.01)

(21) International Application Number:
PCT/US2023/068248

(22) International Filing Date:
09 June 2023 (09.06.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/350,832 09 June 2022 (09.06.2022) US
63/481,257 24 January 2023 (24.01.2023) US

(71) Applicant: **SANTA ANA BIO, INC.** [US/US]; 1105 Atlantic Ave. #104, Alameda, CA 94501 (US).

(72) Inventors: **EMTAGE, Peter**; c/o Santa Ana Bio, Inc., 1105 Atlantic Ave. #104, Alameda, CA 94501 (US). **BOLOTIN, Eugene**; c/o Santa Ana Bio, Inc., 1105 Atlantic Ave. #104, Alameda, CA 94501 (US). **EMTAGE, Nancy**; c/o Santa Ana Bio, Inc., 1105 Atlantic Ave. #104, Alameda, CA 94501 (US).

(74) Agent: **CAI, Yingying** et al.; Goodwin Procter LLP, 100 Northern Avenue, IP Docketing Dept./7th Fl, Boston, MA 02210 (US).

(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, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, 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, ME, 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))*

Published:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) Title: ANTIBODIES TARGETING C-KIT AND/OR SIGLEC AND USES THEREOF

(57) Abstract: Disclosed herein are molecules, such as antibodies and antibody fragments, capable of binding to c-Kit and/or siglec-6.

WO 2023/240272 A2

ANTIBODIES TARGETING C-KIT AND/OR SIGLEC AND USES THEREOF

RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Patent Application No. 63/350,832, filed June 9, 2022; and U.S. Provisional Patent Application No. 63/481,257, filed January 24, 2023, the entire contents of each which are hereby incorporated by reference for all purposes.

BACKGROUND

[0002] Mast cells (MCs) are innate immune cells that play a key role in the inflammatory process. When activated, MCs secrete various immune mediators such as cytokines, leukotrienes, and a large number of proteases into the environment. Mast cell differentiation, growth, and survival are strongly regulated by local tissue environmental factors. Stem cell factor (SCF) and IL-3 are among the best-characterized factors. Undesired mast cell activities have been linked with various diseases or disorders, such as allergies, inflammatory diseases, autoimmune diseases, and cancers.

[0003] c-Kit is a type-III receptor tyrosine kinase and a well-known cell surface receptor that binds to its physiological ligand, SCF. c-Kit is involved in various intracellular signaling pathways and have multiple functions during embryogenesis and adulthood. c-Kit is highly expressed in MCs; upon complete differentiation, MCs rely on c-Kit-dependent signaling for survival, function, and growth. c-Kit antibodies, such as CDX-0158, have been developed.

[0004] Siglec-6 is a member of the CD33-related subfamily of sialic acid-binding immunoglobulin-like lectins (siglecs). Siglec-6 is found on human mast cells, some B cells, and cyto- and syncytiotrophoblasts of the placenta. Siglec-6 has been suggested to possess inhibitory activity for both IgE- and non-IgE-mediated mast cell responses.

[0005] There remains a need for new and improved therapeutics (*e.g.*, for mast cell associated diseases or disorders) that can target c-Kit and/or siglec-6.

SUMMARY

[0006] The present application provides antigen-binding sites that are capable of binding c-Kit and/or siglec-6.

[0007] In one aspects, provided are antibodies or antigen-binding fragments thereof, comprising a first antigen-binding domain that is capable of binding siglec-6 and a second antigen-binding domain that is capable of binding c-Kit.

[0008] In some embodiments, the first antigen-binding domain is capable of binding siglec-6 with a higher affinity than the second antigen-binding domain is capable of binding c-Kit. In some embodiments, the first antigen-binding domain is capable of binding siglec-6 with an affinity characterized by a K_D value that is lower than about 100 uM, lower than about 100 nM, lower than about 10 nM, lower than about 1 nM, lower than about 100 pM, lower than about 10 pM, lower than about 1 pM, lower than about 100 fM, or lower than about 10 fM. In some embodiments, the first antigen-binding domain is capable of binding c-Kit with an affinity characterized by a K_D value that is between about 1 nM and about 900 uM, between about 5 nM and about 500 uM, between about 10 nM and about 100uM, between about 20 nM and about 10 10 uM, or between about 25 and about 500 nM.

[0009] In some embodiments, the antibody or antigen-binding fragment is capable of binding a cell expressing siglect-6.

[0010] In some embodiments, the antibody or antigen-binding fragment is capable of binding a mast cell. In some embodiments, the antibody or antigen-binding fragment binds a mast cell preferentially than another immune cell. In some embodiments, the immune cell is T cell, dendritic cell, macrophage, neutrophil, or basophil.

[0011] In some embodiments, the antibody or antigen-binding fragment thereof does not induce mast cell degranulation.

[0012] In some embodiments, the antibody or antigen-binding fragment thereof has a comparable or lower internalization rate compared to a reference antibody capable of binding siglect-6. In some embodiments, the reference antibody capable of binding siglect-6 comprises a heavy chain variable (VH) region comprising the amino acid sequence of SEQ ID NO: 2, and a light chain variable (VL) region comprising the amino acid sequence of SEQ ID NO: 10.

[0013] In some embodiments, the antibody or antigen-binding fragment thereof has a comparable or lower toxicity compared to a reference antibody capable of binding c-Kit. In some embodiments, the reference antibody capable of binding c-Kit comprises a VH region comprising the amino acid sequence of SEQ ID NO: 40, and a VL region comprising the amino acid sequence of SEQ ID NO: 45.

[0014] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region comprising: heavy chain complementarity-determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 3, or a sequence differing in 1 or 2 amino acids therefrom, HCDR2 having the amino acid sequence of SEQ ID NO: 4 or 21, or a

sequence differing in 1 or 2 amino acids therefrom, and HCDR3 having the amino acid sequence of SEQ ID NO: 5, or a sequence differing in 1 or 2 amino acids therefrom; and

(b) a VL region comprising: heavy chain complementarity-determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 11 or 32, or a sequence differing in 1 or 2 amino acids therefrom, LCDR2 having the amino acid sequence of SEQ ID NO: 12, or a sequence differing in 1 or 2 amino acids therefrom, and LCDR3 having the amino acid sequence of SEQ ID NO: 13, or a sequence differing in 1 or 2 amino acids therefrom.

[0015] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region comprising: HCDR1 having the amino acid sequence of SEQ ID NO: 3, HCDR2 having the amino acid sequence of SEQ ID NO: 4 or 21, and HCDR3 having the amino acid sequence of SEQ ID NO: 5; and

(b) a VL region comprising: LCDR1 having the amino acid sequence of SEQ ID NO: 11 or 32, LCDR2 having the amino acid sequence of SEQ ID NO: 12, and LCDR3 having the amino acid sequence of SEQ ID NO: 13.

[0016] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region comprising:

(i) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 3, 4, and 5, respectively; or

(ii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 3, 21, and 5, respectively; and

(b) a VL region comprising:

(i) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 11, 12, and 13, respectively; or

(ii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 32, 12, and 13, respectively.

[0017] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 2, 20, 23, 25, 27, or 29; and

(b) a VL region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10, 31, 36, or 38.

[0018] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 2, 20, 23, 25, 27, or 29; and

(b) a VL region having the amino acid sequence of SEQ ID NO: 10, 31, 36, or 38.

[0019] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 2, and a VL region having the amino acid sequence of SEQ ID NO: 10;

(b) a VH region having the amino acid sequence of SEQ ID NO: 23, and a VL region having the amino acid sequence of SEQ ID NO: 38;

(c) a VH region having the amino acid sequence of SEQ ID NO: 25, and a VL region having the amino acid sequence of SEQ ID NO: 38;

(d) a VH region having the amino acid sequence of SEQ ID NO: 27, and a VL region having the amino acid sequence of SEQ ID NO: 38; or

(e) a VH region having the amino acid sequence of SEQ ID NO: 29, and a VL region having the amino acid sequence of SEQ ID NO: 38.

[0020] In some embodiments, the first antigen-binding domain comprises: a VH region having the amino acid sequence of SEQ ID NO: 25, and a VL region having the amino acid sequence of SEQ ID NO: 38.

[0021] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 122, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 123, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 124, or a sequence differing in 1 or 2 amino acids therefrom; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 127, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 128, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 129, or a sequence differing in 1 or 2 amino acids therefrom.

[0022] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 122,

HCDR2 having the amino acid sequence of SEQ ID NO: 123, and

HCDR3 having the amino acid sequence of SEQ ID NO: 124; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 127,

LCDR2 having the amino acid sequence of SEQ ID NO: 128, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139.

[0023] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 121; and

(b) a VL region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 126.

[0024] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 121; and

(b) a VL region having the amino acid sequence of SEQ ID NO: 126.

[0025] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 133, 141, or 149, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, 142, 150, or 160, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135, 143, or 151, or a sequence differing in 1 or 2 amino acids therefrom; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 137, 145, 153, or 165, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, 146, or 154, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139, 147, or 155, or a sequence differing in 1 or 2 amino acids therefrom.

[0026] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 133, 141, or 149,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, 142, 150, or 160, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135, 143, or 151; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 137, 145, 153, or 165,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, 146, or 154, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139, 147, or 155.

[0027] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region comprising:

(i) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 133, 134, and 135, respectively;

(ii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 141, 142, and 143, respectively;

(iii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 149, 150, and 151, respectively; or

(iv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 149, 160, and 151, respectively; and

(b) a VL region comprising:

(i) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 137, 138, and 139, respectively;

(ii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 145, 146, and 147, respectively;

(iii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 153, 154, and 155, respectively; or

(iv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 165, 154, and 155, respectively.

[0028] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 132, 140, 148, 159, or 162; and

(b) a VL region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 136, 144, 152, 164, 167, or 169.

[0029] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 132, 140, 148, 159, or 162; and

(b) a VL region having the amino acid sequence of SEQ ID NO: 136, 144, 152, 164, 167, or 169.

[0030] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 132, and a VL region having the amino acid sequence of SEQ ID NO: 139;

(b) a VH region having the amino acid sequence of SEQ ID NO: 140, and a VL region having the amino acid sequence of SEQ ID NO: 144; or

(c) a VH region having the amino acid sequence of SEQ ID NO: 148, and a VL region having the amino acid sequence of SEQ ID NO: 152.

[0031] In some embodiments, the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 159, and a VL region having the amino acid sequence of SEQ ID NO: 164;

(6) a VH region having the amino acid sequence of SEQ ID NO: 162, and a VL region having the amino acid sequence of SEQ ID NO: 167; or

(c) a VH region having the amino acid sequence of SEQ ID NO: 162, and a VL region having the amino acid sequence of SEQ ID NO: 169.

[0032] In some embodiments, the second antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 41, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 42 or 53, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 43, or a sequence differing in 1 or 2 amino acids therefrom; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 46, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 47, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 48, or a sequence differing in 1 or 2 amino acids therefrom.

[0033] In some embodiments, the second antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 41,

HCDR2 having the amino acid sequence of SEQ ID NO: 42 or 53, and

HCDR3 having the amino acid sequence of SEQ ID NO: 43; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 46,

LCDR2 having the amino acid sequence of SEQ ID NO: 47, and

LCDR3 having the amino acid sequence of SEQ ID NO: 48.

[0034] In some embodiments, the second antigen-binding domain comprises a VH region comprising:

(a) HCDR1 having the amino acid sequence of SEQ ID NO: 60, 61, or 62,

(b) HCDR2 having the amino acid sequence of SEQ ID NO: 63, 64, 65, 66, 67, or 68, and

(c) HCDR3 having the amino acid sequence of SEQ ID NO: 69, 70, 71, or 72.

[0035] In some embodiments, the second antigen-binding domain comprises a VH region comprising:

(a) LCDR1 having the amino acid sequence of SEQ ID NO: 90, 91, 92, 93, 94, or 95,

(b) LCDR2 having the amino acid sequence of SEQ ID NO: 96 or 97, and

(c) LCDR3 having the amino acid sequence of SEQ ID NO: 98, 99, 100, 101, 102, 103, or 104.

[0036] In some embodiments, the second antigen-binding domain comprises:

(a) a VH region comprising:

(i) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 42, and 43, respectively;

(ii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 43, respectively;

(iii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 60, 53, and 43, respectively;

(iv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 61, 53, and 43, respectively;

(v) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 62, 53, and 43, respectively;

(vi) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 63, and 43, respectively;

(vii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 64, and 43, respectively;

(viii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 65, and 43, respectively;

(ix) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 66, and 43, respectively;

(x) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 67, and 43, respectively;

(xi) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 68, and 43, respectively;

(xii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 69, respectively;

(xiii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 70, respectively;

(xiv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 71, respectively; or

(xv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 72, respectively; and

(b) a VL region comprising:

(i) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 48, respectively;

(ii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 90, 47, and 48, respectively;

(iii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 91, 47, and 48, respectively;

(iv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 92, 47, and 48, respectively;

(v) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 93, 47, and 48, respectively;

(vi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 94, 47, and 48, respectively;

(vii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 95, 47, and 48, respectively;

(viii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 96, and 48, respectively;

(ix) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 97, and 48, respectively;

(x) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 98, respectively;

(xi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 99, respectively;

(xii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 100, respectively;

(xiii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 101, respectively;

(xiv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 102, respectively;

(xv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 103, respectively; or

(xvi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 104, respectively.

[0037] In some embodiments, the second antigen-binding domain comprises:

(a) a VH region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 40, 52, 55, 57, 59, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, or 85; and

(b) a VL region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 45, 87, 89, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119.

[0038] In some embodiments, the second antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 40, 52, 55, 57, 59, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, or 85; and

(b) a VL region having the amino acid sequence of SEQ ID NO: 45, 87, 89, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119.

[0039] In some embodiments, the second antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 40, and a VL region having the amino acid sequence of SEQ ID NO: 45;

(b) a VH region having the amino acid sequence of SEQ ID NO: 52, and a VL region having the amino acid sequence of SEQ ID NO: 87;

(c) a VH region having the amino acid sequence of SEQ ID NO: 55, and a VL region having the amino acid sequence of SEQ ID NO: 87;

(d) a VH region having the amino acid sequence of SEQ ID NO: 57, and a VL region having the amino acid sequence of SEQ ID NO: 87;

(e) a VH region having the amino acid sequence of SEQ ID NO: 59, and a VL region having the amino acid sequence of SEQ ID NO: 87;

(f) a VH region having the amino acid sequence of SEQ ID NO: 52, and a VL region having the amino acid sequence of SEQ ID NO: 89; or

(g) a VH region having the amino acid sequence of SEQ ID NO: 55, and a VL region having the amino acid sequence of SEQ ID NO: 89.

[0040] In some embodiments, the second antigen-binding domain comprises: a VH region having the amino acid sequence of SEQ ID NO: 52, and a VL region having the amino acid sequence of SEQ ID NO: 87.

[0041] In some embodiments, the antibody or antigen-binding fragment thereof comprises:

(a) a first heavy chain, comprising the VH region of the first antigen-binding domain, and a first heavy chain constant (CH) region or a fragment thereof;

(b) a first light chain, comprising the VL region of the first antigen-binding domain, and a first light chain constant (CL) region or a fragment thereof;

(c) a second heavy chain, comprising the VH region of the second antigen-binding domain, and a second CH region or a fragment thereof; and

(d) a second light chain, comprising the VL region of the second antigen-binding domain, and a second CL region or a fragment thereof.

[0042] In some embodiments, the first and second CH region each comprise an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 130.

[0043] In some embodiments, the first and second heavy chain form a heterodimer, optionally wherein one of the first and second heavy chains comprises a Serine residue at position 366, an Alanine residue at position 368 and a Valine residue at position 407; and the other heavy chain comprises a Tryptophan residue at position 366, wherein the numbering of the constant region is as per the EU index.

[0044] In some embodiments, the first and/or second CH region comprise one or more mutations that reduce or abrogate effector function. In some embodiments, the first and/or second CH region is a human IgG1 CH comprising one of the following mutation(s), wherein the numbering of the constant region is as per the EU index: (a) L234A and/or L235A; (b) A327G, A330S, and/or P331S; (c) E233P, L234V, L235A, and/or G236del; (d) E233P, L234V, and/or L235A; (e) E233P, L234V, L235A, G236del, A327G, A330S, and/or P331S; (f) E233P, L234V, L235A, A327G, A330S, and/or P331S; (g) N297A; (h) N297G; (i) N297Q; (j) L242C, N297C, and/or K334C; (k) A287C, N297G, and/or L306C; (l) R292C, N297G, and/or V302C; (m) N297G, V323C, and/or I332C; (n) V259C, N297G, and/or L306C; (o) L234F, L235Q, K322Q, M252Y, S254T, and/or T256E; (p) L234A, L235A, and/or P329G; or (q) L234A, L235Q, and K322Q. In some embodiments, the first and/or second CH region is a human IgG2 CH comprising one of the following mutation(s), wherein the numbering of the constant region is as per the EU index: (a) A330S and/or P331S; (b) V234A, G237A, P238S, H268A, V309L, A330S, and/or P331S; or (c) V234A, G237A, H268Q, V309L, A330S, P331S, C232S, C233S, S267E, L328F, M252Y, S254T, and/or T256E. In some embodiments, the first and/or second CH region is a human IgG4 CH comprising one of the following mutation(s), wherein the numbering of the constant region is as per the EU index: (a) E233P, F234V, L235A, and/or G236del; (b) E233P, F234V, and/or L235A; (c) S228P and/or L235E; or (d) S228P and/or L235A.

[0045] In some embodiments, the first and/or second CH region comprise one or more mutations that enhance effector function. In some embodiments, the first and/or second CH region is a human IgG1 CH comprising one of the following mutation(s), wherein the numbering of the constant region is as per the EU index: (a) F243L, R292P, Y300L, V305I, and/or P396L; (b) S239D and/or I332E; (c) S239D, I332E, and/or A330L; (d) S298A, E333A, and/or K334A; (e) G236A, S239D, and/or I332E; (f) K326W and/or E333S; (g) S267E, H268F, and/or S324T; or (h) E345R, E430G, and/or S440Y.

[0046] In some embodiments, at least one of the first and second heavy chains is non-fucosylated. In some embodiments, the antibody or antigen-binding fragment thereof is produced in a cell line (a) having an alpha-1,6-fucosyltransferase (Fut8) knockout; or (b) line overexpressing β1,4-N-acetylglucosaminyltransferase III (GnT-III) and optionally overexpressing Golgi μ- mannosidase II (ManII).

[0047] In some embodiments, the first and/or second CL region is a human kappa constant region.

[0048] In some embodiments,

(a) (i) the first antigen-binding domain comprises:

a VH region comprising HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 3, 21, and 5, respectively; and

a VL region comprising LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 32, 12, and 13, respectively; and

(ii) the second antigen-binding domain comprises:

a VH region comprising HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 43, respectively; and

a VL region comprising LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 48, respectively; or

(b) (i) the first antigen-binding domain comprises:

a VH region comprising HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 122, 123, and 124, respectively; and

a VL region comprising LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 127, 128, and 129, respectively; and

(ii) the second antigen-binding domain comprises:

a VH region comprising HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 43, respectively; and

a VL region comprising LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 48, respectively; or

[0049] In some embodiments, (a) the first antigen-binding domain comprises a VH region having the amino acid sequence of SEQ ID NO: 25, and a VL region having the amino acid sequence of SEQ ID NO: 38; and the second antigen-binding domain comprises a VH region having the amino acid sequence of SEQ ID NO: 52, and a VL region having the amino acid sequence of SEQ ID NO: 87; or

(b) the first antigen-binding domain comprises a VH region having the amino acid sequence of SEQ ID NO: 121, and a VL region having the amino acid sequence of SEQ ID NO: 126; and the second antigen-binding domain comprises a VH region having the amino acid sequence of SEQ ID NO: 52, and a VL region having the amino acid sequence of SEQ ID NO: 87.

[0050] In some embodiments, the antigen-binding fragment is a Fab, a Fab', a Fab2, a F(ab')2, Fv, a single-chain Fv (scFv), or a diabody.

[0051] In some embodiments, the antibody or antigen-binding fragment thereof is conjugated to an agent. In some embodiments, agent is a cytotoxic agent or label.

[0052] In another aspect, provided are compositions comprising the antibody or antigen-binding fragment thereof described above. In some embodiments, the antibody or antigen-binding fragment comprises an Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, optionally wherein less than 50% of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some embodiments, substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue.

[0053] In another aspect, provided are antibodies or antigen binding fragments thereof that is capable of binding to siglec-6, comprising:

(a) a heavy chain variable (VH) region comprising:

heavy chain complementarity-determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 133, 141, or 149, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, 142, 150, or 160, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135, 143, or 151, or a sequence differing in 1 or 2 amino acids therefrom; and

(b) a light chain variable (VL) region comprising:

light chain complementarity-determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 137, 145, 153, or 165, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, 146, or 154, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139, 147, or 155, or a sequence differing in 1 or 2 amino acids therefrom.

[0054] In some embodiments, the antibody or antigen binding fragment comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 133, 141, or 149,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, 142, 150, or 160, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135, 143, or 151; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 137, 145, 153, or 165,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, 146, or 154, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139, 147, or 155.

[0055] In some embodiments, the antibody or antigen binding fragment comprises:

(a) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 133, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135, or a sequence differing in 1 or 2 amino acids therefrom; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 137, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139, or a sequence differing in 1 or 2 amino acids therefrom; or

(b) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 141, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 142, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 143, or a sequence differing in 1 or 2 amino acids therefrom; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 145, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 146, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 147, or a sequence differing in 1 or 2 amino acids therefrom; or

(c) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 149, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 150 or 160, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 151, or a sequence differing in 1 or 2 amino acids therefrom; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 153 or 165, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 154, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 155, or a sequence differing in 1 or 2 amino acids therefrom.

[0056] In some embodiments, the antibody or antigen binding fragment comprises:

(a) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 133,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 137,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139; or

(b) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 141,

HCDR2 having the amino acid sequence of SEQ ID NO: 142, and

HCDR3 having the amino acid sequence of SEQ ID NO: 143; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 145,

LCDR2 having the amino acid sequence of SEQ ID NO: 146, and

LCDR3 having the amino acid sequence of SEQ ID NO: 147; or

(c) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 149,

HCDR2 having the amino acid sequence of SEQ ID NO: 150 or 160, and

HCDR3 having the amino acid sequence of SEQ ID NO: 151; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 153 or 165,

LCDR2 having the amino acid sequence of SEQ ID NO: 154, and

LCDR3 having the amino acid sequence of SEQ ID NO: 155.

[0057] In some embodiments, the antibody or antigen binding fragment comprises:

(a) a VH region comprising:

(i) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 133, 134, and 135, respectively;

(ii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 141, 142, and 143, respectively;

(iii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 149, 150, and 151, respectively; or

(iv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 149, 160, and 151, respectively; and

(b) a VL region comprising:

(i) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 137, 138, and 139, respectively;

(ii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 145, 146, and 147, respectively;

(iii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 153, 154, and 155, respectively; or

(iv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 165, 154, and 155, respectively.

[0058] In some embodiments, the VH region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 132, 140, 148, 159, or 162; and the VL region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 136, 144, 152, 164, 167, or 169.

[0059] In some embodiments, the VH region comprises the amino acid sequence of SEQ ID NO: 132, 140, 148, 159, or 162; and the VL region comprises the amino acid sequence of SEQ ID NO: 136, 144, 152, 164, 167, or 169.

[0060] In some embodiments, (a) the VH region comprises the amino acid sequence of SEQ ID NO: 132, and the VL region comprises the amino acid sequence of SEQ ID NO: 139; (b) the VH region comprises the amino acid sequence of SEQ ID NO: 140, and the VL region

comprises the amino acid sequence of SEQ ID NO: 144; or (c) the VH region comprises the amino acid sequence of SEQ ID NO: 148, and the VL region comprises the amino acid sequence of SEQ ID NO: 152.

[0061] In some embodiments, (a) the VH region comprises the amino acid sequence of SEQ ID NO: 159, and the VL region comprises the amino acid sequence of SEQ ID NO: 164; (b) the VH region comprises the amino acid sequence of SEQ ID NO: 162, and the VL region comprises the amino acid sequence of SEQ ID NO: 167; or (c) the VH region comprises the amino acid sequence of SEQ ID NO: 162, and the VL region comprises the amino acid sequence of SEQ ID NO: 169.

[0062] In some embodiments, the antibody is a humanized antibody.

[0063] In another aspect, provided are antibodies or antigen binding fragments thereof that is capable of binding to siglec-6, comprising:

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

heavy chain complementarity-determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 3,

HCDR2 having the amino acid sequence of SEQ ID NO: 21, and

HCDR3 having the amino acid sequence of SEQ ID NO: 5; and

(a) a light chain variable domain (VL) comprising:

lilght chain complementarity-determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 32,

LCDR2 having the amino acid sequence of SEQ ID NO: 12, and

LCDR3 having the amino acid sequence of SEQ ID NO: 13.

[0064] In some embodiments, the VH region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 20, 23, 25, 27, or 29; and the VL region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 31, 36, or 38.

[0065] In some embodiments, the antibody or antigen binding fragment comprises: the VH region comprises the amino acid sequence of SEQ ID NO: 20, 23, 25, 27, or 29; and the VL region comprisesthe amino acid sequence of SEQ ID NO: 31, 36, or 38.

[0066] In some embodiments, (a) the VH region comprises the amino acid sequence of SEQ ID NO: 23, and the VL region comprises the amino acid sequence of SEQ ID NO: 38; (b) the VH region comprises the amino acid sequence of SEQ ID NO: 25, and the VL region comprises the amino acid sequence of SEQ ID NO: 38;

(c) the VH region comprises the amino acid sequence of SEQ ID NO: 27, and the VL region comprises the amino acid sequence of SEQ ID NO: 38; or

(d) the VH region comprises the amino acid sequence of SEQ ID NO: 29, and the VL region comprises the amino acid sequence of SEQ ID NO: 38.

[0067] In some embodiments, the VH region comprises the amino acid sequence of SEQ ID NO: 25, and the VL region comprises the amino acid sequence of SEQ ID NO: 38.

[0068] In some embodiments, the antibody or antigen binding fragment is capable of binding siglec-6 with a K_D value of at most about 10 nM, at most about 5 nM, or at most about 1 nM. In some embodiments, the antibody or antigen binding fragment thereof (a) is capable of binding human siglec-6; (b) is capable of binding cynomolgus siglec-6; (c) is capable of binding a mast cell, optionally preferentially than another immune cell (e.g., T cell, dendritic cell, macrophage, neutrophil, or basophil); (d) does not induce mast cell degranulation; and/or (e) has a comparable or lower internalization rate compared to a reference antibody capable of binding siglect-6, optionally wherein the reference antibody comprises a VH region comprising the amino acid sequence of SEQ ID NO: 2 and a VL region comprising the amino acid sequence of SEQ ID NO: 10.

[0069] In another aspect, provided are humanized antibodies or antigen binding fragments thereof that is capable of binding to c-Kit, comprising:

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

heavy chain complementarity-determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 41, or a sequence differing in 1 amino acid therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 53, or a sequence differing in 1 amino acid therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 43, or a sequence differing in 1 amino acid therefrom; and

(a) a light chain variable domain (VL) comprising:

lilght chain complementarity-determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 46, or a sequence differing in 1 amino acid therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 47, or a sequence differing in 1 amino acid therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 48, or a sequence differing in 1 amino acid therefrom.

[0070] In some embodiments, the antibody or antigen binding fragment comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 41,

HCDR2 having the amino acid sequence of SEQ ID NO: 53, and

HCDR3 having the amino acid sequence of SEQ ID NO: 43; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 46,

LCDR2 having the amino acid sequence of SEQ ID NO: 47, and

LCDR3 having the amino acid sequence of SEQ ID NO: 48.

[0071] In some embodiments, the VH comprises:

(a) HCDR1 having the amino acid sequence of SEQ ID NO: 60, 61, or 62,

(b) HCDR2 having the amino acid sequence of SEQ ID NO: 63, 64, 65, 66, 67, or 68, and

(c) HCDR3 having the amino acid sequence of SEQ ID NO: 69, 70, 71, or 72.

[0072] In some embodiments, the VL comprises:

(a) LCDR1 having the amino acid sequence of SEQ ID NO: 90, 91, 92, 93, 94, or 95,

(b) LCDR2 having the amino acid sequence of SEQ ID NO: 96 or 97, and

(c) LCDR3 having the amino acid sequence of SEQ ID NO: 98, 99, 100, 101, 102, 103, or 104.

[0073] In some embodiments,

(a) the VH region comprises:

(i) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 43, respectively;

(ii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 60, 53, and 43, respectively;

(iii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 61, 53, and 43, respectively;

(iv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 62, 53, and 43, respectively;

(v) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 63, and 43, respectively;

(vi) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 64, and 43, respectively;

(vii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 65, and 43, respectively;

(viii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 66, and 43, respectively;

(ix) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 67, and 43, respectively;

(x) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 68, and 43, respectively;

(xi) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 69, respectively;

(xii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 70, respectively;

(xiii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 71, respectively; or

(xiv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 72, respectively; and

(b) the VL region comprising:

(i) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 48, respectively;

(ii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 90, 47, and 48, respectively;

(iii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 91, 47, and 48, respectively;

(iv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 92, 47, and 48, respectively;

(v) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 93, 47, and 48, respectively;

(vi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 94, 47, and 48, respectively;

(vii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 95, 47, and 48, respectively;

(viii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 96, and 48, respectively;

(ix) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 97, and 48, respectively;

(x) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 98, respectively;

(xi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 99, respectively;

(xii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 100, respectively;

(xiii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 101, respectively;

(xiv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 102, respectively;

(xv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 103, respectively; or

(xvi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 104, respectively.

[0074] In some embodiments, the VH region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 52, 55, 57, 59, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, or 85; and the VL region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 87, 89, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119.

[0075] In some embodiments, the VH region comprises the amino acid sequence of SEQ ID NO: 52, 55, 57, 59, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, or 85; and the VL region comprisesthe amino acid sequence of SEQ ID NO: 87, 89, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119.

[0076] In some embodiments,

(a) the VH region comprises the amino acid sequence of SEQ ID NO: 40, and the VL region comprises the amino acid sequence of SEQ ID NO: 45;

(b) the VH region comprises the amino acid sequence of SEQ ID NO: 52, and the VL region comprises the amino acid sequence of SEQ ID NO: 87;

(c) the VH region comprises the amino acid sequence of SEQ ID NO: 55, and the VL region comprises the amino acid sequence of SEQ ID NO: 87;

(d) the VH region comprises the amino acid sequence of SEQ ID NO: 57, and the VL region comprises the amino acid sequence of SEQ ID NO: 87;

(e) the VH region comprises the amino acid sequence of SEQ ID NO: 59, and the VL region comprises the amino acid sequence of SEQ ID NO: 87;

(f) the VH region comprises the amino acid sequence of SEQ ID NO: 52, and the VL region comprises the amino acid sequence of SEQ ID NO: 89; or

(g) the VH region comprises the amino acid sequence of SEQ ID NO: 55, and the VL region comprises the amino acid sequence of SEQ ID NO: 89.

[0077] In some embodiments, the VH region comprises the amino acid sequence of SEQ ID NO: 52, and the VL region comprises the amino acid sequence of SEQ ID NO: 87.

[0078] In some embodiments, the antibody or antigen binding fragment thereof is capable of binding siglec-6 with a K_D value of between about 10 and about 500 nM, between about 20 and about 100 nM; or between about 25 and about 80 nM. In some embodiments, the antibody or antigen binding fragment thereof (a) is capable of binding human c-Kit; (b) is capable of binding cynomolgus c-Kit; and/or (c) has a comparable or lower toxicity compared to a reference antibody capable of binding c-Kit, optionally wherein the reference antibody comprises a VH region comprising the amino acid sequence of SEQ ID NO: 40, and a VL region comprising the amino acid sequence of SEQ ID NO: 45.

[0079] In some embodiments, the antigen binding fragment described above is a Fab, a F(ab')2, a Fab', a single-chain Fv (scFv), an Fv fragment, a Fd fragment, or a diabody.

[0080] In some embodiments, the antibody or antigen binding fragment thereof comprises an antibody heavy chain constant region, optionally wherein the constant region is a human IgG heavy chain constant region, optionally wherein the human IgG heavy chain constant region is an IgG1 heavy chain constant region. In some embodiments, the constant region comprises one or more mutations that reduce or abrogate effector function. In some embodiments, the constant region comprises one or more mutations that enhance effector function. In some embodiments, at least one of the first and second heavy chains is afucosylated (e.g., at least 75% afucosylated).

[0081] In some embodiments, the antibody or antigen binding fragment described herein is conjugated to a therapeutic agent or a label.

[0082] In another aspects, provided are polynucleotides encoding antibodies or antigen binding fragmenst thereof descrived herein. In another aspects, provided are expression vectors comprising the polynucleotide. In another aspects, provided are host cells comprising the polynucleotide or the expression vector. In some embodiments, the host cell is a mammalian or insect cell. In some embodiments, the host cell (a) comprises a Fut8 knockout; and/or (b) overexpresses GnT-III and optionally ManII.

[0083] In another aspects, provided are pharmaceutical compositions comprising an antibody or antigen binding fragment thereof described herein.

[0084] In another aspects, provided are methods of treating a disease or disorder in a subject in need thereof, comprising administering to the subject an effective amount of an antibody or antigen binding fragment thereof described herein or a pharmaceutical composition described herein. In some embodiments, the disease or disorder is a mast cell associated disease or disorder. In some embodiments, the disease or disorder is an autoimmune disease, an allergy, an inflammation, pruritus, or a cancer. In some embodiments, the disease or disorder is associated with expression of siglec-6 and/or c-Kit.

BRIEF DESCRIPTION OF THE DRAWINGS

[0085] **FIG. 1** depicts the inhibition of stem cell factor (SCF)-dependent cell proliferation in M-07e cells by SR1 c-Kit antibodies.

[0086] **FIG. 2** depicts the inhibition of SCF-dependent cell proliferation in M-07e cells by c-Kit/siglec 6 bispecific antibodies.

[0087] **FIG. 3** shows that humanized siglec-6 antibody hRND-3 with normal, effector slient, or effector enhanced Fc domains did not induce degranulation of mast cells as measured by β-hexosaminidase release. IFNγ treatment of mast cells to upregulate FcγRI did not lead to degranulation by siglec-6 antibodies. Treatment with FcεRIα crosslinking antibody plus SCF and PMA/Ionomycin were used as positive controls.

DETAILED DESCRIPTION

Definitions

[0088] The terms "**a**" and "**an**" as used herein mean "one or more" and include the plural unless the context is inappropriate.

[0089] As used herein, the terms "**about**," "**approximately**," and "**comparable to**," when used herein in reference to a value, refer to a value that is similar to the referenced value in the context of that referenced value. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variance encompassed by "about," "approximately," and "comparable to" in that context. For example, in some embodiments, the terms "about," "approximately," and "comparable to" may encompass a range of values that within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of the referred value.

[0090] As used herein, the terms "**antagonistic**," "**neutralizing**" or "**blocking**," when used in reference to an antibody or antigen-binding fragment thereof, is intended to refer to an antibody or fragment thereof whose binding to its target results in inhibition of at least some of the biological activity of the target.

[0091] As used herein, "**antibody**" refers to a polypeptide whose amino acid sequence includes immunoglobulins and fragments thereof which specifically bind to a designated antigen, or fragments thereof. Antibodies in accordance with the present invention may be of any type (e.g., IgA, IgD, IgE, IgG, or IgM) or subtype (e.g., IgA1, IgA2, IgG1, IgG2, IgG3, or IgG4). Those of ordinary skill in the art will appreciate that a characteristic sequence or portion of an antibody may include amino acids found in one or more regions of an antibody (*e.g.*, variable region, hypervariable region, constant region, heavy chain, light chain, and combinations thereof). Moreover, those of ordinary skill in the art will appreciate that a characteristic sequence or portion of an antibody may include one or more polypeptide chains, and may include sequence elements found in the same polypeptide chain or in different polypeptide chains.

[0092] As used herein, "**antibody mimetic**" refers to any molecule that is capable of mimicking an antibody's ability to bind an antigen, but which are not limited to antibody structures. Examples of antibody mimetics include, but are not limited to, Affibodies, Affilins, Affimers, Afftins, Alphabodies, Anticalins, avimers, Centyrins, DARPins, Fynomers, monobodies, and nanoCLAMPs.

[0093] An "**antigen-binding fragment**" of an antibody comprises a portion of an intact antibody, which portion is still capable of antigen binding. In certain embodiments, papain digestion of antibodies produce two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire light chain along with the variable region domain of the heavy chain (V_H), and the first constant domain of one heavy chain (C_H1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. In certain embodiments, pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and that is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the C_H1 domain, including one or more cysteines from the antibody hinge region. Fab'-SH designates an Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab'

fragments having hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0094] As used herein, the term "**antigen-binding site**" refers to any molecule or any part of a molecule that is capable of antigen binding. In human antibodies, the antigen-binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains are referred to as "hypervariable regions" which are interposed between more conserved flanking stretches known as "**framework regions**," or "**FR**." Thus, the term "FR" refers to amino acid sequences which are naturally found between and adjacent to hypervariable regions in immunoglobulins. In a human antibodies, 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. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "**complementarity-determining regions**," or "**CDRs**." In certain animals, such as camels and cartilaginous fish, the antigen-binding site may by formed by a single antibody chain providing a "single domain antibody." An antigen-binding site can also be or exist in an antibody mimetic.

[0095] The CDRs can be determined by the methods described in Kabat et al., J. Biol. Chem. 252, 6609-6616 (1977) and Kabat et al., Sequences of protein of immunological interest. (1991), Chothia et al., J. Mol. Biol. 196:901-917 (1987), and MacCallum et al., J. Mol. Biol. 262:732-745 (1996). The CDRs determined under these definitions typically include overlapping or subsets of amino acid residues when compared against each other. In certain embodiments, the term "CDR" is a CDR as defined by MacCallum et al., J. Mol. Biol. 262:732-745 (1996) and Martin A., Protein Sequence and Structure Analysis of Antibody Variable Domains, in Antibody Engineering, Kontermann and Dubel, eds., Chapter 31, pp. 422-439, Springer-Verlag, Berlin (2001). In certain embodiments, the term "CDR" is a CDR as defined by Kabat et al., J. Biol. Chem. 252, 6609-6616 (1977) and Kabat et al., Sequences of protein of immunological interest. (1991). In certain embodiments, heavy chain CDRs and light chain CDRs of an antibody are defined using different conventions. For example, in certain embodiments, the heavy chain CDRs are defined according to MacCallum (*supra*), and the light CDRs are defined according to Kabat (*supra*). CDRH1, CDRH2 and CDRH3 denote the heavy chain CDRs, and CDRL1, CDRL2 and CDRL3 denote the light chain CDRs.

[0096] As used herein, "**binding affinity**" of a molecule of the disclosure (*e.g.*, an antigent-binding site) to a given targets (*e.g.*, FCER1 or c-Kit) can be determined by a multitude of methods known to those skilled in the art. Such methods include, but are not limited to, fluorescence titration, ELISA (enzyme-linked immunosorbent assay), calorimetric methods such as isothermal titration calorimetry (ITC), and surface plasmon resonance (SPR).

[0097] As used herein, "**bispecific**" refers to a molecule is able to specifically bind to at least two distinct targets. Typically, a bispecific molecule comprises two antigen-binding sites, each of which is specific for a different target. In some embodiments, a bispecific molecule is capable of binding two targets simultaneously.

[0098] As used herein, unless otherwise specified, "**c-Kit**" means human c-Kit defined by UniProt P10721, and includes variants, isoforms, and species homologs of human c-Kit. c-Kit is also known as "KIT," "CD117," "mast/stem cell growth factor receptor Kit," "SCFR," "proto-oncogene c-Kit," or "tyrosine-protein kinase Kit," which are used interchangeably.

[0099] As used herein, "**detectable affinity**" refers to the ability to bind to a given target with an affinity constant, typically measured by K_D or EC_{50}, of at most about 10^{-5} M or lower (a lower K_D or EC_{50} value reflects better binding ability). Lower affinities that are no longer measurable with common methods such as ELISA (enzyme-linked immunosorbent assay) are of secondary importance.

[0100] As used herein, the term "**epitope**" is an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule, known as the paratope, and which is comprised of the six complementary-determining regions of the antibody. A single antigen may have more than one epitope. Epitopes may be conformational or linear. A conformational epitope is comprised of spatially juxtaposed amino acids from different segments of a linear polypeptide chain. A linear epitope is comprised of adjacent amino acid residues in a polypeptide chain.

[0101] An "**Fc**" fragment comprises the carboxy-terminal portions of both heavy chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

[0102] A "**fragment**" with respect to an antigen (e.g., siglec-6 or c-Kit) refers to N-terminally and/or C- terminally truncated or protein domains of the antigen. In some embodiments, a fragment of an antigen retains the capability of the full-length antigen to be

recognized and/or bound by an antibody or antigen-binding fragment thereof of the disclosure.

[0103] As used herein, "**fucosylation**" or "**fucosylated**" refers to the presence of fucose residues within the oligosaccharides attached to the peptide backbone of an antibody. Specifically, a fucosylated antibody comprises α(l,6)-linked fucose at the innermost N-acetylglucosamine (GlcNAc) residue in one or both of the N-linked oligosaccharides attached to the antibody Fc region. "**Non-fucosylated**," "**afucosylated**," or "**fucose-deficient**" antibody refers to a glycosylation antibody variant comprising an Fc region wherein a carbohydrate structure attached to the Fc region has reduced fucose or lacks fucose. In some embodiments, non-fucosylated or fucose-deficient antibodies have reduced fucose relative to the amount of fucose on the same antibody produced in a cell line. In some embodiments, an antibody with reduced fucose or lacking fucose has improved ADCC function.

[0104] The "**degree of fucosylation**" is the percentage of fucosylated oligosaccharides relative to all oligosaccharides, which may be identified by methods known in the art, e.g., in an N-glycosidase F treated antibody composition assessed by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS). In a composition of a "**fully fucosylated antibody**," essentially all oligosaccharides comprise fucose residues, i.e., are fucosylated. In some embodiments, a composition of a fully fucosylated antibody has a degree of fucosylation of at least about 90%. In contrast, in a composition of a "**fully non-fucosylated antibody**," essentially none of the oligosaccharides are fucosylated. In some embodiments, a composition of a fully non-fucosylated antibody has a degree of fucosylation of less than about 10%. In a composition of a "**partially fucosylated antibody**," only part of the oligosaccharides comprise fucose. An individual antibody in such a composition may comprise fucose residues in none, one or both of the N-linked oligosaccharides in the Fc region, provided that the composition does not comprise essentially all individual antibodies that lack fucose residues in the N-linked oligosaccharides in the Fc region, nor essentially all individual antibodies that contain fucose residues in both of the N-linked oligosaccharides in the Fc region. In one embodiment, a composition of a partially fucosylated antibody has a degree of fucosylation of about 10% to about 80% (e.g., about 50% to about 80%, about 60% to about 80%, or about 70% to about 80%). Assays for measuring degree of fucosylation, as well as methods and cell lines for producing antibodies with altered, reduced, or eliminated fucosylation, are known in the art (see, e.g., WO 2023/044390 A1).

[0105] As used herein, the term "**monoclonal antibody**" refers to an antibody obtained from a population of substantially homogeneous antibodies, *e.g.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, monoclonal antibodies may be made by a hybridoma method, or may be made by recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567). "Monoclonal antibodies" may also be isolated from phage antibody libraries.

[0106] As used herein, the term "**pharmaceutical composition**" refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*.

[0107] As used herein, the term "**pharmaceutically acceptable salt**" refers to any pharmaceutically acceptable salt (*e.g.*, acid or base) of a compound described in the present application which, upon administration to a subject, is capable of providing a compound described in this application or an active metabolite or residue thereof. As is known to those of skill in the art, "salts" of the compounds described in the present application may be derived from inorganic or organic acids and bases.

[0108] Exemplary acids include, but are not limited to, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic, benzenesulfonic acid, and the like. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds described in the present application and their pharmaceutically acceptable acid addition salts.

[0109] Exemplary bases include, but are not limited to, alkali metal (*e.g.*, sodium) hydroxides, alkaline earth metal (*e.g.*, magnesium) hydroxides, ammonia, and compounds of formula NW_4^+, wherein W is C_{1-4} alkyl, and the like.

[0110] Exemplary salts include, but are not limited to: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate,

camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, undecanoate, and the like. Other examples of salts include anions of the compounds described in the present application compounded with a suitable cation such as Na^+, NH_4^+, and NW_4^+ (wherein W is a C_{1-4} alkyl group), and the like.

[0111] For therapeutic use, salts of the compounds described in the present application are contemplated as being pharmaceutically acceptable. However, salts of acids and bases that are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

[0112] As used herein, unless otherwise specified, "**siglec-6**" means human siglec-6 defined by UniProt O43699, and includes variants, isoforms, and species homologs of human siglec-6. Siglec-6 is also known as "SIGLEC6," "sialic acid-binding Ig-like lectin 6," "CD33 antigen-like 1," "CD33L," "CD33L1," "CD33L2," "CD327," "CDw327," "obesity-binding protein 1," or "OB-BP1," which are used interchangeably.

[0113] As used herein, the terms "**subject**" and "**patient**" refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably include humans.

[0114] As used herein, the phrases "**therapeutically effective amount**" and "**effective amount**" are used interchangeably and refer to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount may vary according to factors such as the type of disease (*e.g.*, cancer), disease state, age, sex, and/or weight of the individual, and the ability of an immunoconjugate (or pharmaceutical composition thereof) to elicit a desired response in the individual. An effective amount may also be an amount for which any toxic or detrimental effects of the immunoconjugate or pharmaceutical composition thereof are outweighed by therapeutically beneficial effects.

[0115] As used herein, to "**treat**" a condition or "**treatment**" of the condition (*e.g.*, the conditions described herein such as cancer) is an approach for obtaining beneficial or desired results, such as clinical results. Beneficial or desired results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions; diminishment of

extent of disease, disorder, or condition; stabilized (*i.e.*, not worsening) state of disease, disorder, or condition; preventing spread of disease, disorder, or condition (*e.g.*, of a primary cancer and/or of a secondary metastases); delay or slowing the progress of the disease, disorder, or condition; amelioration or palliation of the disease, disorder, or condition; and remission (whether partial or total), whether detectable or undetectable.

Antigen-binding sites

Antigen-binding sites capable of binding c-Kit

[0116] In another aspect, the present application provides antigen-binding sites that are capable of binding c-Kit.

[0117] In some embodiments, provided are antigen-binding sites capable of binding c-Kit, present as antibody mimetics. In some embodiments, provided are antigen-binding sites that are variants of an antigen-binding site capable of binding c-Kit and present as an antibody mimetic described herein, in that such antigen-binding sites comprise an amino acid sequence that is at least 85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of the antigen-binding site capable of binding c-Kit and present as an antibody mimetic described herein.

[0118] In some embodiments, provided are antigen-binding sites capable of binding c-Kit, present as antibodies (e.g., bispecific antibodies). In some embodiments, provided are antigen-binding sites that are variants of an antigen-binding site capable of binding c-Kit and present as an antibody described herein, in that such antigen-binding sites comprise an amino acid sequence that is at least 85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence comprised in said antigen-binding site capable of binding c-Kit and present as an antibody described herein.

[0119] In some embodiments, provided antigen-binding sites capable of binding c-Kit comprise a heavy chain variable domain (VH) comprising a CDR-H1, CDR-H2, and CDR-H3, and/or a light chain variable domain (VL) comprising a CDR-L1, CDR-L2, and CDR-L3. In some embodiments, provided antigen-binding sites capable of binding c-Kit comprise a VH and a VL, wherein the VH comprises a CDR-H1, CDR-H2, and CDR-H3, and the VL comprises a CDR-L1, CDR-L2, and CDR-L3, and wherein the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 are each independently selected from those of a VH or VL described in **Table 10**. CDRs are determined under Kabat, AbM, Chothia, or any other CDR

determination method known in the art, of the VH and VL. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 each independently selected from those described in **Table 10**. In some embodiments, provided are antigen-binding sites that are variants of the antigen-binding sites capable of binding c-Kit described herein, in that such antigen-binding sites have CDR sequences that each differ by no more than two amino acid residues (e.g., two or one amino acid residue(s)) from a CDR sequence described in **Table 10**. In some embodiments, provided are an antigen-binding sites that are variants of the antigen-binding sites capable of binding c-Kit described herein, in that such antigen-binding sites have a set of six CDRs whose sequences collectively differ by no more than two amino acid residues (e.g., two or one amino acid residues) from a set of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 selected from **Table 10**. In some embodiments, provided are an antigen-binding sites that are variants of the antigen-binding sites capable of binding c-Kit described herein, in that such antigen-binding sites have a set of six CDRs whose sequences collectively differ by no more than two amino acid residues (e.g., two or one amino acid residues) from those of an anti-c-Kit having a set of VH and VL selected from **Table 10**.

[0120] In some embodiments, provided antigen-binding sites capable of binding c-Kit comprise a VH sequence as described in **Table 10**, and a VL sequence as described in **Table 10**. In some embodiments, provided are antigen-binding sites that are variants of the antigen-binding site capable of binding c-Kit described herein, such as an anti-c-Kit having a set of VH and VL selected from **Table 10**, in that such antigen-binding site have (1) a VH comprising an amino acid sequence that is at least 85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of a VH described in **Table 10**; and (2) a VL comprising an amino acid sequence that is at least 85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of a VL described in **Table 10**.

[0121] In some embodiments, provided antigen-binding sites capable of binding c-Kit comprise a heavy chain sequence as described in **Table 10**, and a light chain sequence as described in **Table 10**. In some embodiments, provided are antigen-binding sites that are variants of the antigen-binding site capable of binding c-Kit described herein, such as an anti-c-Kit having a set of heavy chain and light chain selected from **Table 10**, in that such antigen-binding site have (1) a heavy chain comprising an amino acid sequence that is at least

85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of a heavy chain described in **Table 10**; and (2) a light chain comprising an amino acid sequence that is at least 85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of a light chain described in **Table 10**.

[0122] In certain embodiments, an antigen-binding site described in the present application is derived from murine SR1. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 40, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 45. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 40 and 45, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, comprising the amino acid sequences of SEQ ID NOs: 41, 42, and 43, respectively. In certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 46, 47, and 48, respectively. In certain embodiments, the antigen-binding site comprises (a) a VH that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 41, 42, and 43, respectively; and (b) a VL that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 46, 47, and 48, respectively.

[0123] In certain embodiments, an antigen-binding site described in the present application is derived from humanized SR1-1. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 52, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 87. In certain embodiments, the VH comprises an amino acid sequence of SEQ ID NO: 52, 55, 57,

59, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, or 85. In certain embodiments, the VL comprises an amino acid sequence of SEQ ID NO: 87, 89, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 52 and 87, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, comprising the amino acid sequences of SEQ ID NOs: 41, 53, and 43, respectively. In certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 46, 47, and 48, respectively. In certain embodiments, the antigen-binding site comprises (a) a VH that comprises CDR-H1, CDR-H2, and CDR-H3comprising the amino acid sequences of SEQ ID NOs: 41, 53, and 43, respectively; and (b) a VL that comprises CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 46, 47, and 48, respectively.

[0124] In certain embodiments, a humanized SR1-1 variant comprises CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, the sequence of each of which differs by no more than one amino from CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 52 and 87, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, the sequence of each of which differs by no more than one amino from SEQ ID NOs: 41, 53, and 43, respectively. In certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3, the sequence of each of which differs by no more than one amino from SEQ ID NOs: 46, 47, and 48, respectively. In certain embodiments, a humanized SR1-1 variant comprises CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, whose sequences collectively differ by at least one amino acid residue (e.g., one, two, or three) from CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 52 and 87, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, whose sequences collectively differ by at least one amino acid residue from SEQ ID NOs: 41, 53, and 43, respectively. In certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3, whose sequences collectively differ by at least one amino acid residue from SEQ ID NOs: 46, 47, and 48, respectively. In certain embodiments, CDR-H1 comprises the amino acid sequence of SEQ ID NO: 60, 61, or 62. In certain embodiments, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 63, 64, 65, 66, 67, or 68. In

certain embodiments, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 69, 70, 71, or 72. In certain embodiments, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 90, 91, 92, 93, 94, or 95. In certain embodiments, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 96 or 97. In certain embodiments, CDR-L3 comprises the amino acid sequence of SEQ ID NO: 98, 99, 100, 101, 102, 103, or 104.

[0125] In certain embodiments, an antigen-binding site described in the present application is derived from humanized SR1-2. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 55, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 87. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 55 and 87, respectively.

[0126] In certain embodiments, an antigen-binding site described in the present application is derived from humanized SR1-3. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 57, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 87. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 57 and 87, respectively.

[0127] In certain embodiments, an antigen-binding site described in the present application is derived from humanized SR1-4. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the

amino acid sequence of SEQ ID NO: 59, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 87. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 59 and 87, respectively.

[0128] In certain embodiments, an antigen-binding site described in the present application is derived from humanized SR1-5. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 52, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 89. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 52 and 89, respectively.

[0129] In certain embodiments, an antigen-binding site described in the present application is derived from humanized SR1-6. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 55, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 89. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 55 and 89, respectively.

[0130] In each of the foregoing embodiments, it is contemplated herein that the VH and/or VL sequences that bind c-Kit may contain amino acid alterations (e.g., at least 1, 2, 3, 4, 5, or 10 amino acid substitutions, deletions, or additions) in the framework regions of the

VH and/or VL without affecting their ability to c-Kit. For example, it is contemplated herein that VH and VL sequences that bind c-Kit may contain cysteine heterodimerization mutations, facilitating formation of a disulfide bridge between the VH and VL to form an scFv.

[0131] In certain embodiments, antigen-binding sites disclosed herein bind human c-Kit at a K_D value less than or equal to (affinity greater than or equal to) 900 uM, 500 uM, 250 uM, 100 uM, 50 uM, 25 uM, 10 uM, 9 uM, 8 uM, 7 uM, 6 uM, 5 uM, 4 uM, 3 uM, 2 uM, 1 uM, 900 nM, 800 nM, 700 nM, 600 nM, 500 nM, 400 nM, 300 nM, 200 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 45 nM, 40 nM, 35 nM, 30 nM, 25 nM, 20 nM, 15 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, 90 pM, 80 pM, 70 pM, 60 pM, 50 pM, 45 pM, 40 pM, 35 pM, 30 pM, 25 pM, 20 pM, 15 pM, 10 pM, 9 pM, 8 pM, 7 pM, 6 pM, 5 pM, 4 pM, 3 pM, 2 pM, or 1 pM. In certain embodiments, an antigen-binding site of the present application binds human c-Kit at a K_D value higher than or equal to (affinity lower than or equal to) 1 pM, 2 pM, 3 pM, 4 pM, 5 pM, 6 pM, 7 pM, 8 pM, 9 pM 10 pM, 15 pM, 20 pM, 25 pM, 30 pM, 35 pM, 40 pM, 45 pM, 50 pM, 60 pM, 70 pM, 80 pM, 90 pM, 0.1 nM, 0.2 nM, 0.3 nM, 0.4 nM, 0.5 nM, 0.6 nM, 0.7 nM, 0.8 nM, 0.9 nM, 1 nM, 2 nM, 3 nM, 4 nM, 5 nM, 6 nM, 7 nM, 8 nM, 9 nM, 10 nM, 15 nM, 20 nM, 25 nM, 30 nM, 35 nM, 40 nM, 45 nM, 50 nM, 60 nM, 70 nM, 80 nM, 90 nM, 100 nM, 150 nM, 200 nM, 250 nM, 300 nM, 400 nM, 500 nM, 600 nM, 700 nM, 800 nM, 900 nM, 1 uM, 2 uM, 3 uM, 4 uM, 5 uM, 6 uM, 7 uM, 8 uM, 9 uM, 10 uM, 25 uM, 50 uM, 100 uM, 250 uM, 500 uM, or 900 uM . In certain embodiments, the antigen binding site of the present application binds human c-Kit at a K_D value in the range of picomolar to micromolar. In certain embodiments, the antigen binding site of the present application binds human c-Kit at a K_D value in the range of about 1 pM to about 900 uM, 1 pM to about 500 uM, 1 pM to about 100 uM, 1 pM to about 50 uM, 1 pM to about 10 uM, 1 pM to about 5 uM, 1 pM to about 1 uM, 1 pM to about 500 nM, 1 pM to about 100 nM, 1 pM to about 50 nM, 1 pM to about 10 nM, 1 pM to about 5 nM, 1 pM to about 1 nM, 1 pM to about 500 pM, 1 pM to about 100 pM, 1 pM to about 50 pM, 1 pM to about 10 pM, 1 pM to about 5 pM, 5 pM to about 900 uM, 5 pM to about 500 uM, 5 pM to about 100 uM, 5 pM to about 50 uM, 5 pM to about 10 uM, 5 pM to about 5 uM, 5 pM to about 1 uM, 5 pM to about 500 nM, 5 pM to about 100 nM, 5 pM to about 50 nM, 5 pM to about 10 nM, 5 pM to about 5 nM, 5 pM to about 1 nM, 5 pM to about 500 pM, 5 pM to about 100 pM, 5 pM to about 50 pM, 5 pM to about 10 pM, 10 pM to about 900 uM, 10 pM to about 500 uM, 10 pM to about 100 uM, 10 pM to about 50 uM, 10 pM to about 10 uM, 10 pM to about 5 uM, 10 pM to about 1 uM, 10

pM to about 500 nM, 10 pM to about 100 nM, 10 pM to about 50 nM, 10 pM to about 10 nM, 10 pM to about 5 nM, 10 pM to about 1 nM, 10 pM to about 500 pM, 10 pM to about 100 pM, 10 pM to about 50 pM, 50 pM to about 900 uM, 50 pM to about 500 uM, 50 pM to about 100 uM, 50 pM to about 50 uM, 50 pM to about 10 uM, 50 pM to about 5 uM, 50 pM to about 1 uM, 50 pM to about 500 nM, 50 pM to about 100 nM, 50 pM to about 50 nM, 50 pM to about 10 nM, 50 pM to about 5 nM, 50 pM to about 1 nM, 50 pM to about 500 pM, 50 pM to about 100 pM, 100 pM to about 900 uM, 100 pM to about 500 uM, 100 pM to about 100 uM, 100 pM to about 50 uM, 100 pM to about 10 uM, 100 pM to about 5 uM, 100 pM to about 1 uM, 100 pM to about 500 nM, 100 pM to about 100 nM, 100 pM to about 50 nM, 100 pM to about 10 nM, 100 pM to about 5 nM, 100 pM to about 1 nM, 100 pM to about 500 pM, 500 pM to about 500 uM, 500 pM to about 100 uM, 500 pM to about 50 uM, 500 pM to about 10 uM, 500 pM to about 5 uM, 500 pM to about 1 uM, 500 pM to about 500 nM, 500 pM to about 100 nM, 500 pM to about 50 nM, 500 pM to about 10 nM, 500 pM to about 5 nM, 500 pM to about 1 nM, 1 nM to about 500 uM, 1 nM to about 100 uM, 1 nM to about 50 uM, 1 nM to about 10 uM, 1 nM to about 5 uM, 1 nM to about 1 uM, 1 nM to about 500 nM, 1 nM to about 100 nM, 1 nM to about 50 nM, 1 nM to about 10 nM, 1 nM to about 5 nM, 5 nM to about 100 uM, 5 nM to about 50 uM, 5 nM to about 10 uM, 5 nM to about 5 uM, 5 nM to about 1 uM, 5 nM to about 500 nM, 5 nM to about 100 nM, 5 nM to about 50 nM, 5 nM to about 10 nM, 10 nM to about 10 uM, 10 nM to about 5 uM, 10 nM to about 1 uM, 10 nM to about 500 nM, 10 nM to about 100 nM, 10 nM to about 50 nM, 50 nM to about 10 uM, 50 nM to about 5 uM, 50 nM to about 1 uM, 50 nM to about 500 nM, 50 nM to about 100 nM, 100 nM to about 10 uM, 100 nM to about 5 uM, 100 nM to about 1 uM, 100 nM to about 500 nM, 500 nM to about 10 uM, 500 nM to about 5 uM, 500 nM to about 1 uM, 1 uM to about 10 uM, 1 uM to about 5 uM, or 5 uM to about 10 uM.. In some embodiments, K_D values are measured using standard binding assays, for example, bio-layer interferometry.

[0132] In some embodiments, antigen-binding sites disclosed herein bind cynomolgus c-Kit at a comparable affinity (K_D value) to that of binding human c-Kit. In some embodiments, the affinity of the antigen-binding site to cynomolgus c-Kit relative to its affinity to human c-Kit, as measured in the same assay, is within a 2-fold, 3-fold, 5-fold, or 10-fold difference (either with greater affinity to human c-Kit or with greater affinity to cynomolgus c-Kit).

[0133] In some embodiments, antigen-binding sites disclosed herein bind c-Kit expressed on cell surface.

[0134] In some embodiments, antigen-binding sites disclosed herein does not significantly bind other c-Kit family members. In some embodiments, antigen-binding sites disclosed herein does not significantly bind PDGFRa, PDGFRb, CSF1R, or FLT3.

Antigen-binding sites capable of binding siglec-6

[0135] In another aspect, the present application provides antigen-binding sites that are capable of binding siglec-6.

[0136] In some embodiments, provided are antigen-binding sites capable of binding siglec-6, present as antibody mimetics. In some embodiments, provided are antigen-binding sites that are variants of an antigen-binding site capable of binding siglec-6 and present as an antibody mimetic described herein, in that such antigen-binding sites comprise an amino acid sequence that is at least 85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of the antigen-binding site capable of binding siglec-6 and present as an antibody mimetic described herein.

[0137] In some embodiments, provided are antigen-binding sites capable of binding siglec-6, present as antibodies (e.g., bispecific antibodies). In some embodiments, provided are antigen-binding sites that are variants of an antigen-binding site capable of binding siglec-6 and present as an antibody described herein, in that such antigen-binding sites comprise an amino acid sequence that is at least 85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence comprised in said antigen-binding site capable of binding siglec-6 and present as an antibody described herein.

[0138] In some embodiments, provided antigen-binding sites capable of binding siglec-6 comprise a heavy chain variable domain (VH) comprising a CDR-H1, CDR-H2, and CDR-H3, and/or a light chain variable domain (VL) compriseing a CDR-L1, CDR-L2, and CDR-L3.

[0139] In some embodiments, provided antigen-binding sites capable of binding siglec-6 comprise a VH and a VL, wherein the VH comprises a CDR-H1, CDR-H2, and CDR-H3, and the VLcomprises a CDR-L1, CDR-L2, and CDR-L3, wherein the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 are each independently selected from those of a VH or VL described in **Table 9**. CDRs are determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1,

CDR-L2, and CDR-L3 each independently selected from those described in **Table 9**. In some embodiments, provided are antigen-binding sites that are variants of the antigen-binding sites capable of binding siglec-6 described herein, in that such antigen-binding sites have CDR sequences that each differ by no more than two amino acid residues (e.g., two or one amino acid residue(s)) from a CDR sequence described in **Table 9**. In some embodiments, provided are an antigen-binding sites that are variants of the antigen-binding sites capable of binding siglec-6 described herein, in that such antigen-binding sites have a set of six CDRs whose sequences collectively differ by no more than two amino acid residues (e.g., two or one amino acid residues) from a set of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 selected from **Table 9**. In some embodiments, provided are an antigen-binding sites that are variants of the antigen-binding sites capable of binding siglec-6 described herein, in that such antigen-binding sites have a set of six CDRs whose sequences collectively differ by no more than two amino acid residues (e.g., two or one amino acid residues) from those of an anti-siglec-6 having a set of VH and VL selected from **Table 9**.

[0140] In some embodiments, provided antigen-binding sites capable of binding siglec-6 comprise a VH sequence as described in **Table 9**, and a VL sequence as described in **Table 9**. In some embodiments, provided are antigen-binding sites that are variants of the antigen-binding site capable of binding siglec-6 described herein, such as an anti-siglec-6 having a set of VH and VL selected from **Table 9**, in that such antigen-binding site have (1) a VH comprising an amino acid sequence that is at least 85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of a VH described in **Table 9**; and (2) a VL domain comprising an amino acid sequence that is at least 85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of a VL described in **Table 9**.

[0141] In some embodiments, provided antigen-binding sites capable of binding siglec-6 comprise a heavy chain sequence as described in **Table 9**, and a light chain sequence as described in **Table 9**. In some embodiments, provided are antigen-binding sites that are variants of the antigen-binding site capable of binding siglec-6 described herein, such as an anti-siglec-6 having a set of heavy chain and light chain selected from **Table 9**, in that such antigen-binding site have (1) a heavy chain comprising an amino acid sequence that is at least 85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid

sequence of a heavy chain described in **Table 9**; and (2) a light chain comprising an amino acid sequence that is at least 85%, at least 87.5%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of a light chain described in **Table 9**.

[0142] In certain embodiments, an antigen-binding site described in the present application is derived from murine RND. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 2, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 10. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 2 and 10, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, comprising the amino acid sequences of SEQ ID NOs: 3, 4, and 5, respectively. In certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 11, 12, and 13, respectively. In certain embodiments, the antigen-binding site comprises (a) a VH that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 3, 4, and 5, respectively; and (b) a VL that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 11, 12, and 13, respectively.

[0143] In certain embodiments, an antigen-binding site described in the present application is derived from humanized RND-2. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 23, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 38. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 23 and

38, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, comprising the amino acid sequences of SEQ ID NOs: 3, 21, and 5, respectively. In certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 32, 12, and 13, respectively. In certain embodiments, the antigen-binding site comprises (a) a VH that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 3, 21, and 5, respectively; and (b) a VL that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 32, 12, or 13, respectively.

[0144] In certain embodiments, an antigen-binding site described in the present application is derived from humanized RND-3. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 25, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 38. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 25 and 38, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, comprising the amino acid sequences of SEQ ID NOs: 3, 21, and 5, respectively.

[0145] In certain embodiments, an antigen-binding site described in the present application is derived from humanized RND-4. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 27, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 38. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 27 and 38, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, comprising the amino acid sequences of SEQ ID NOs: 3, 4, and 5, respectively. In

certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 32, 12, and 13, respectively. In certain embodiments, the antigen-binding site comprises (a) a VH that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 3, 4, and 5, respectively; and (b) a VL that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 32, 12, or 13, respectively.

[0146] In certain embodiments, an antigen-binding site described in the present application is derived from humanized RND-5. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 29, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 38. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 29 and 38, respectively.

[0147] In certain embodiments, an antigen-binding site described in the present application is derived from JML-1. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 121, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 126. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 121 and 126, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, comprising the amino acid sequences of SEQ ID NOs: 122, 123, and 124, respectively. In certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 127, 128, and 129, respectively. In certain embodiments, the antigen-binding site comprises (a) a VH that comprises CDR1,

CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 122, 123, and 124, respectively; and (b) a VL that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 127, 128, and 129, respectively.

[0148] In certain embodiments, an antigen-binding site described in the present application is derived from murine M2. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 132, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 136. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 132 and 136, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, comprising the amino acid sequences of SEQ ID NOs: 133, 134, and 135, respectively. In certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 137, 138, and 139, respectively. In certain embodiments, the antigen-binding site comprises (a) a VH that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 133, 134, and 135, respectively; and (b) a VL that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 137, 138, and 139, respectively.

[0149] In certain embodiments, an antigen-binding site described in the present application is derived from murine M7. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 140, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 144. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 140 and 144, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and

CDR-H3, comprising the amino acid sequences of SEQ ID NOs: 141, 142, and 143, respectively. In certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 145, 146, and 147, respectively. In certain embodiments, the antigen-binding site comprises (a) a VH that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 141, 142, and 143, respectively; and (b) a VL that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 145, 146, and 147, respectively.

[0150] In certain embodiments, an antigen-binding site described in the present application is derived from murine M11. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 148, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 152. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 148 and 152, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, comprising the amino acid sequences of SEQ ID NOs: 149, 150, and 151, respectively. In certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 153, 154, and 155, respectively. In certain embodiments, the antigen-binding site comprises (a) a VH that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 149, 150, and 151, respectively; and (b) a VL that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 153, 154, and 155, respectively.

[0151] In certain embodiments, an antigen-binding site described in the present application is derived from humanized M11-1. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 159, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 164. In

certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 159 and 164, respectively. In certain embodiments, the VH comprises CDR-H1, CDR-H2, and CDR-H3, comprising the amino acid sequences of SEQ ID NOs: 149, 160, and 151, respectively. In certain embodiments, the VL comprises CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs: 165, 154, and 155, respectively. In certain embodiments, the antigen-binding site comprises (a) a VH that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 149, 160, and 151, respectively; and (b) a VL that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 165, 154, and 155, respectively.

[0152] In certain embodiments, an antigen-binding site described in the present application is derived from humanized M11-2. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 162, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 167. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 162 and 167, respectively.

[0153] In certain embodiments, an antigen-binding site described in the present application is derived from humanized M11-3. For example, in certain embodiments, an antigen-binding site described in the present application comprises a VH that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 162, and a VL that comprises an amino acid sequence at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 169. In certain embodiments, the antigen-binding site comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, determined under Kabat, AbM, Chothia, or any other CDR

determination method known in the art, of the VH and VL sequences of SEQ ID NOs: 162 and 169, respectively.

[0154] In certain embodiments, antigen-binding sites disclosed herein bind human siglec-6 at a K_D value less than or equal to (affinity greater than or equal to) 900 nM, 800 nM, 700 nM, 600 nM, 500 nM, 400 nM, 300 nM, 200 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 45 nM, 40 nM, 35 nM, 30 nM, 25 nM, 20 nM, 15 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, 90 pM, 80 pM, 70 pM, 60 pM, 50 pM, 40 pM, 30 pM, 20 pM, 10 pM, 9 pM, 8 pM, 7 pM, 6 pM, 5 pM, 4 pM, 3 pM, 2 pM, 1 pM, 0.9 pM, 0.8 pM, 0.7 pM, 0.6 pM, 0.5 pM, 0.4 pM, 0.3 pM, 0.2 pM, 0.1 pM, 90 fM, 80 fM, 70 fM, 60 fM, 50 fM, 40 fM, 30 fM, 20 fM, 10 fM, 9 fM, 8 fM, 7 fM, 6 fM, 5 fM, 4 fM, 3 fM, 2 fM, or 1 fM. In certain embodiments, antigen-binding sites disclosed herein bind human siglec-6 at a K_D value less than or equal to (affinity greater than or equal to) 10 nM. In certain embodiments, antigen-binding sites disclosed herein bind human siglec-6 at a K_D value less than or equal to (affinity greater than or equal to) 0.1 nM, 0.2 nM, 0.3 nM, 0.4 nM, 0.5 nM, 0.6 nM, 0.7 nM, 0.8 nM, 0.9 nM, 1.0 nM, 1.1 nM, 1.2 nM, 1.3 nM, 1.4 nM, 1.5 nM, 1.6 nM, 1.7 nM, 1.8 nM, 1.9 nM, 2.0 nM, 2.1 nM, 2.2 nM, 2.3 nM, 2.4 nM, 2.5 nM, 2.6 nM, 2.7 nM, 2.8 nM, 2.9 nM, 3.0 nM, 3.1 nM, 3.2 nM, 3.3 nM, 3.4 nM, 3.5 nM, 3.6 nM, 3.7 nM, 3.8 nM, 3.9 nM, 4.0 nM, 4.1 nM, 4.2 nM, 4.3 nM, 4.4 nM, 4.5 nM, 4.6 nM, 4.7 nM, 4.8 nM, 4.9 nM, or 5.0 nM. In certain embodiments, the antigen binding site of the present application binds human siglec-6 at a K_D value in the range femtomolar to nanomolar. In certain embodiments, the antigen binding site of the present application binds human siglec-6 at a K_D value in the range of about 1 fM to about 900 nM, 1 fM to about 500 nM, 1 fM to about 100 nM, 1 fM to about 50 nM, 1 fM to about 10 nM, 1 fM to about 5 nM, 1 fM to about 1 nM, 1 fM to about 500 pM, 1 fM to about 100 pM, 1 fM to about 50 pM, 1 fM to about 10 pM, 1 fM to about 5 pM, 1 fM to about 1 pM, 1 fM to about 500 fM, 1 fM to about 100 fM, 1 fM to about 50 fM, 1 fM to about 10 fM, 1 fM to about 5 fM, 5 fM to about 900 nM, 5 fM to about 500 nM, 5 fM to about 100 nM, 5 fM to about 50 nM, 5 fM to about 10 nM, 5 fM to about 5 nM, 5 fM to about 1 nM, 5 fM to about 500 pM, 5 fM to about 100 pM, 5 fM to about 50 pM, 5 fM to about 10 pM, 5 fM to about 5 pM, 5 fM to about 1 pM, 5 fM to about 500 fM, 5 fM to about 100 fM, 5 fM to about 50 fM, 5 fM to about 10 fM, 10 fM to about 900 nM, 10 fM to about 500 nM, 10 fM to about 100 nM, 10 fM to about 50 nM, 10 fM to about 10 nM, 10 fM to about 5 nM, 10 fM to about 1 nM, 10 fM to about 500 pM, 10 fM to about 100 pM, 10 fM to about 50 pM, 10 fM to about 10 pM, 10 fM to about 5 pM, 10 fM to about 1 pM, 10 fM to about 500 fM, 10 fM to about 100 fM, 10 fM to about 50 fM, 50

fM to about 900 nM, 50 fM to about 500 nM, 50 fM to about 100 nM, 50 fM to about 50 nM, 50 fM to about 10 nM, 50 fM to about 5 nM, 50 fM to about 1 nM, 50 fM to about 500 pM, 50 fM to about 100 pM, 50 fM to about 50 pM, 50 fM to about 10 pM, 50 fM to about 5 pM, 50 fM to about 1 pM, 50 fM to about 500 fM, 50 fM to about 100 fM, 100 fM to about 900 nM, 100 fM to about 500 nM, 100 fM to about 100 nM, 100 fM to about 50 nM, 100 fM to about 10 nM, 100 fM to about 5 nM, 100 fM to about 1 nM, 100 fM to about 500 pM, 100 fM to about 100 pM, 100 fM to about 50 pM, 100 fM to about 10 pM, 100 fM to about 5 pM, 100 fM to about 1 pM, 100 fM to about 500 fM, 500 fM to about 900 nM, 500 fM to about 500 nM, 500 fM to about 100 nM, 500 fM to about 50 nM, 500 fM to about 10 nM, 500 fM to about 5 nM, 500 fM to about 1 nM, 500 fM to about 500 pM, 500 fM to about 100 pM, 500 fM to about 50 pM, 500 fM to about 10 pM, 500 fM to about 5 pM, 500 fM to about 1 pM, 1 pM to about 900 nM, 1 pM to about 500 nM, 1 pM to about 100 nM, 1 pM to about 50 nM, 1 pM to about 10 nM, 1 pM to about 5 nM, 1 pM to about 1 nM, 1 pM to about 500 pM, 1 pM to about 100 pM, 1 pM to about 50 pM, 1 pM to about 10 pM, 1 pM to about 5 pM, 5 pM to about 10 nM, 5 pM to about 5 nM, 5 pM to about 1 nM, 5 pM to about 500 pM, 5 pM to about 100 pM, 5 pM to about 50 pM, 5 pM to about 10 pM, 10 pM to about 10 nM, 10 pM to about 5 nM, 10 pM to about 1 nM, 10 pM to about 500 pM, 10 pM to about 100 pM, 10 pM to about 50 pM, 50 pM to about 10 nM, 50 pM to about 5 nM, 50 pM to about 1 nM, 50 pM to about 500 pM, 50 pM to about 100 pM, 100 pM to about 10 nM, 100 pM to about 5 nM, 100 pM to about 1 nM, 100 pM to about 500 pM, 500 pM to about 10 nM, 500 pM to about 5 nM, 500 pM to about 1 nM, 1 nM to about 10 nM, 1 nM to about 5 nM, or 5 nM to about 10 nM. In some embodiments, K_D values are measured using standard binding assays, for example, bio-layer interferometry.

[0155] In some embodiments, antigen-binding sites disclosed herein bind cynomolgus siglec-6 at a comparable affinity (K_D value) to that of binding human siglec-6. In some embodiments, the affinity of the antigen-binding site to cynomolgus siglec-6 relative to its affinity to human siglec-6, as measured in the same assay, is within a 2-fold, 3-fold, 5-fold, or 10-fold difference (either with greater affinity to human siglec-6 or with greater affinity to cynomolgus siglec-6).

[0156] In some embodiments, antigen-binding sites disclosed herein bind siglec-6expressed on cell surface. In some embodiments, antigen-binding sites disclosed herein bind mast cells. In some embodiments, antigen-binding sites disclosed herein bind mast cells referentially to other immune cells (e.g., T cells, dendritic cells, macrophages, neutrophils, or basophils).

[0157] In some embodiments, antigen-binding sites disclosed herein does not significantly bind other siglec-6 family members. In some embodiments, antigen-binding sites disclosed herein does not significantly bind siglec 1-5, 7-12, 14, or 15.

[0158] In some embodiments, antigen-binding sites disclosed herein do not induce mass cell degranulation.

Molecules containing antigen-binding sites

[0159] Also provided herein are molecules containing disclosed antigen-binding sites. Such molecules may be, but are not limited to, antibodies or antigen-binding fragments thereof, antibody fragments, nanobodies, antibody mimetics, etc.

[0160] In some embodiments, provided are antibodies or antigen-binding fragments thereof containing disclosed antigen-binding sites. In some embodiments, the antibodies or antigen-binding fragments thereof are multispecific, e.g., bispecific. In some embodiments, the antibodies are monoclonal antibodies. In some embodiments, the antibodies are humanized antbodies. In some embodiments, the antibodies are human antbodies. In some embodiments, the antibodies are non-fucosylated (afucosylated). In some embodiments, the antibodies are at most about 90%, at most about 80%, at most about 70%, at most about 60%, at most about 50%, at most about 40%, at most about 30%, at most about 25%, at most about 20%, at most about 15%, at most about 10%, at most about 5%, at most about 4%, at most about 3%, at most about 2%, at most about 1% fucosylated. In some embodiments, the antibodies are afucosylated. In some embodiments, the antibodies are at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% afucosylated.

[0161] In some embodiments, the antibodies or antigen-binding fragments thereof are capable of binding c-Kit. In some embodiments, the antibodies or antigen-binding fragments thereof are capable of binding siglec-6. In some embodiments, the antibodies or antigen-binding fragments thereof are capable of binding c-Kit and siglec-6.

[0162] In some embodiments, provided are antibody fragments thereof containing disclosed antigen-binding sites. For example, the fragments may be, but not limited to, an Fab, an scFab (single-chain Fab), an $F(ab')_2$, a Fab', a single-chain Fv (scFv), an Fv fragment, or a diabody (a dimer of scFv). In some embodiments, the antibody fragments are multispecific, e.g., bispecific. In some embodiments, the antibody fragments are capable of

binding c-Kit. In some embodiments, the antibody fragments are capable of binding siglec-6. In some embodiments, the antibody fragments are capable of binding c-Kit and siglec-6.

[0163] In some embodiments, provided are antibody mimetics containing disclosed antigen-binding sites. For example, the fragments may be, but not limited to, an Affibody, an Affilin, an Affimer, an afftin, an Alphabody, an Anticalin, an avimer, a Centyrin, a DARPin, a Fynomer, a monobody, or a nanoCLAMP. In some embodiments, the antibody mimetics are multispecific, e.g., bispecific. In some embodiments, the antibody mimetics are capable of binding c-Kit. In some embodiments, the antibody mimetics are capable of binding siglec-6. In some embodiments, the antibody mimetics are capable of binding c-Kit and siglec-6.

[0164] In some embodiments, provided are bispecific molecules comprising two antigen-binding sites disclosed herein.

[0165] In some embodiments, the bispecific molecule comprises an antigen-binding site capable of binding c-Kit described herein. In some embodiments, the bispecific molecule comprises an antigen-binding site capable of binding siglec-6 described herein. In some embodiments, the bispecific molecule comprises an antigen-binding site capable of binding c-Kit described herein and an antigen-binding site capable of binding siglec-6 described herein.

[0166] In some embodiments, provided are bispecific molecules comprising: (i) an antigen-binding site capable of binding siglec-6 described herein; and (ii) an antigen-binding site capable of binding c-Kit described herein. In some embodiments, the bispecific molecule comprises a first antigen-binding site comprising the VH region and the VL region of an antigen-binding site capable of binding siglec-6 described herein and a second antigen-binding site comprising the VH region and the VL region of an antigen-binding site capable of binding c-Kit described herein. In some embodiments, the first antigen-binding site (that is capable of binding siglec-6) comprises a VH region and a VL region which is, or which is derived from, the VH/VL region of murine RND, humanized RND, or JML-1, as described herein; and the second antigen-binding domain (that is capable of binding c-Kit) comprises a VH region and a VL region which is, or which is derived from, the VH/VL region of muthe SR1 or humanized SR1, as described herein.

[0167] In some embodiments, the bispecific molecule is capable of binding a cell expressing siglect-6. In some embodiments, the bispecific molecule is capable of binding a cell expressing c-Kit. In some embodiments, the bispecific molecule is capable of binding a mast cell. In some embodiments, the bispecific molecule binds a mast cell preferentially than another immune cell (e.g., T cell, dendritic cell, macrophage, neutrophil, or basophil). In some embodiments, the bispecific molecule has a comparable or lower internalization rate

compared to a reference antigen-binding site capable of binding siglect-6 (e.g., an antibody comprising a VH and a VL of SEQ ID NOs: 2 and 10, respectively). In some embodiments, the bispecific molecule has a comparable or lower toxicity compared to a reference antigen-binding site capable of binding c-Kit (e.g., an antibody comprising a VH and a VL of SEQ ID NOs: 40 and 45, respectively).

[0168] Various formats and uses of bispecific molecules are known in the art. Bispecific molecules according to the present invention are not limited to any particular bispecific format or method of producing it.

[0169] In some embodiments, the bispecific molecule may be a bispecific antibody or a fragment or derivative thereof including, but not limited to, (i) a single antibody that has two arms, each comprising a different antigen-binding site; (ii) a bispecific scFv, *e.g.*, via two scFvs linked by a peptide linker; (iii) a dual-variable-domain antibody (DVD-Ig), where each light chain and heavy chain contains two variable domains in tandem through a short peptide linkage; (iv) a bispecific $(Fab')_2$ fragment; (v) a diabody; (vi) a tandem diabody (TandAb), a fusion of two diabodies; and (vii) a "dock-and-lock (DNL)-Fab3, a trivalent bispecific binding protein consisting of two identical Fab fragments linked to a different Fab fragment; (viii) IgG-like molecules with engineered Fc to force heterodimerization; (ix) recombinant IgG-like dual targeting molecules, wherein the two sides of the molecule each contain the Fab fragment or part of the Fab fragment of at least two different antibodies; (x) IgG fusion molecules, wherein full length IgG antibodies are fused to extra Fab fragment or parts of Fab fragment; (xi) Fc fusion molecules; (v) Fab fusion molecules; and (vi) scFv- and diabody-based and heavy chain antibodies (e.g., domain antibodies, nanobodies) wherein different scFvs, diabodies, or heavy-chain antibodies (*e.g.*, domain antibodies, nanobodies) are fused to each other or to another protein or carrier molecule fused to heavy-chain constant-domains, Fc-regions or parts thereof.

[0170] Examples of IgG-like molecules with engineered Fc include, but are not limited, to the triomab, knobs-into-holes (kih) molecules (e.g., kih IgG with common light chain), CrossMAbs, orthoFab IgG molecules electrostatically-matched molecules, the LUZ-Y molecules, DIG-body and PIG-body molecules, the Strand Exchange Engineered Domain body (SEEDbody) molecules, the Biclonics molecules, FcΔAdp molecules, bispecific IgG1 and IgG2 molecules, Azymetric scaffold molecules, and the DuoBody molecules. Examples of recombinant IgG-like dual targeting molecules include, but are not limited, to Dual Targeting (DT)-Ig molecules, two-in-one antibody, mAb^2, and Zybody. Examples of IgG fusion molecules include, but are not limited to, DVD-Ig molecules and IgG-scFv. Examples

of Fc fusion molecules include, but are not limited to, scFv/Fc fusions, SCORPION molecules, and Fc-DART molecules. Examples of Fab fusion bispecific antibodies include, but are not limited to, $F(ab)_2$ molecules, DNL molecules, and Fab-Fv molecules. Examples of scFv- and diabody-based and domain antibodies include, but are not limited, to bispecific T cell engager (BiTE) molecules, tandem diabody molecules (TandAb), dual-affinity retargeting technology (DART) molecules, single-chain diabody molecules, TCR-like antibodies (AIT, ReceptorLogics), human serum albumin scfv fusion, COMBODY molecules, dual targeting nanobodies, and dual targeting heavy chain only domain antibodies.

[0171] In ceratin embodiments, disclosed bispecific molecules may be fusion proteins containing one or more antibody mimetics. In ceratin embodiments, disclosed bispecific molecules may be fusion proteins containing one or more antibodies or antigen-binding fragments thereof. In some embodiments, disclosed bispecific molecule may be fusion proteins containing one or more antibody mimetics and one or more antibodies or antigen-binding fragments thereof.

[0172] In some embodiments, molecules containing disclosed antigen-binding sites further comprise antibody constant regions or fragments or variants thereof. In some embodiments, the antibody constant region may be, *e.g.*, a 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 certain embodiments, the antibody constant region or fragment or variant thereof has an amino acid sequence at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to a heavy chain constant region of, *e.g.*, the heavy chain constant region of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, or IgE, preferably, *e.g.*, the heavy chain constant region of human IgG1, IgG2, IgG3, or IgG4. In certain embodiments, the antibody constant region may be 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/or 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 an 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.

[0173] In some embodiments, the constant region comprises one or more mutation(s) that reduce effector function. For example, in certain embodiments, the constant region comprises a heavy chain constant region of IgG1, comprising one or more of the following mutations, numbering based on EU index, (a) L234A and/or L235A; (b) A327G, A330S, and/or P331S; (c) E233P, L234V, L235A, and/or G236del; (d) E233P, L234V, and/or L235A; (e) E233P, L234V, L235A, G236del, A327G, A330S, and/or P331S; (f) E233P, L234V, L235A, A327G, A330S, and/or P331S; (g) N297A; (h) N297G; (i) N297Q; (j) L242C, N297C, and/or K334C; (k) A287C, N297G, and/or L306C; (l) R292C, N297G, and/or V302C; (m) N297G, V323C, and/or I332C; (n) V259C, N297G, and/or L306C; (o) L234F, L235Q, K322Q, M252Y, S254T, and/or T256E; (p) L234A, L235A, and/or P329G; or (q) L234A, L235Q, and K322Q. In certain embodiments, the constant region comprises a heavy chain constant region of IgG2, comprising one or more of the following mutations, numbering based on EU index, (a) A330S and/or P331S; (b) V234A, G237A, P238S, H268A, V309L, A330S, and/or P331S; or (c) V234A, G237A, H268Q, V309L, A330S, P331S, C232S, C233S, S267E, L328F, M252Y, S254T, and/or T256E. In certain embodiments, the constant region comprises a heavy chain constant region of IgG4, comprising one or more of the following mutations, numbering based on EU index, (a) E233P, F234V, L235A, and/or G236del; (b) E233P, F234V, and/or L235A; (c) S228P and/or L235E; or (d) S228P and/or L235A.

[0174] In some embodiments, the constant region comprises one or more mutation(s) that enhance effector function. For example, in certain embodiments, the constant region comprises a heavy chain constant region of IgG1, comprising one or more of the following mutations, numbering based on EU index, (a) F243L, R292P, Y300L, V305I, and/or P396L; (b) S239D and/or I332E; (c) S239D, I332E, and/or A330L; (d) S298A, E333A, and/or K334A; (e) G236A, S239D, and/or I332E; (f) K326W and/or E333S; (g) S267E, H268F, and/or S324T; or (h) E345R, E430G, and/or S440Y.

[0175] In some embodiments, a disclosed antigen-binding site is linked to an antibody constant region or fragment or variant thereof. In certain embodiments, the antigen-binding site is linked to an IgG constant region including hinge, CH_2 and CH_3 domains with or without a CH_1 domain.

[0176] In some embodiments, molecules containing disclosed antigen-binding sites further comprises a Fc region that is non-fucosylated. In some embodiments, the Fc region is non-fucosylated IgG1 Fc region.

Amino acid sequence modifications

[0177] Amino acid sequence modification(s) of the antigen-binding sites and molecules containing antigen-binding sites (e.g., antibodies or antigen-binding fragments thereof) disclosed herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibodies or antigen-binding fragments. Amino acid sequence variants can be prepared, *e.g.*, by introducing appropriate nucleotide changes into a nucleic acid sequence encoding the antigen-binding site or molecule containing antigen-binding site, or by peptide synthesis. Such modifications can include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences. Any combination of deletion, insertion, and substitution can be made, provided that the antigen-binding site or molecule containing antigen-binding site retains the desired characteristics and/or functions. In some embodiments, amino acid changes are introduced to alter post-translational processes, such as changing the number or position of glycosylation sites.

[0178] A useful method for identification of certain residues or regions that are preferred locations for mutagenesis is called "alanine scanning mutagenesis." In this method, a residue or group of target residues are identified (*e.g.*, charged residues such as Arg, Asp, His, Lys, and Glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis may be conducted at the target codon or region, and the expressed variants may be screened for a desired activity.

[0179] Examples of amino acid sequence insertions include, but are not limited to, amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. An example of a terminal insertion includes, but are not limited to, N-terminal methionyl residues.

[0180] In some embodiments, the antigen-binding site or molecule containing antigen-binding site is fused at one terminus to another polypeptide, *e.g.*, a cytotoxic polypeptide, an enzyme, or a polypeptide which increases the serum half-life of the antibody or antigen-binding fragment.

[0181] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the amino acid sequence of the molecule replaced by a different residue. Sites of greatest interest for substitutional mutagenesis are typically the hypervariable regions, but framework region alterations are also contemplated. Examples of conservative substitutions are shown in **Table 1** under the heading of "preferred substitutions." More substantial changes, under the heading "exemplary substitutions" in **Table 1**, or as further described below in reference to amino acid classes, may be introduced and the resulting antibodies or antigen-binding fragments screened.

Table 1. Exemplary Amino acid substitutions

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

[0182] Substantial modifications in the biological properties of the antibody may be accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are typically divided into groups based on common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr;

(3) acidic: Asp, Glu;

(4) basic: Asn, Gln, His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro; and

(6) aromatic: Trp, Tyr, Phe.

[0183] Non-conservative substitutions can entail exchanging a member of one of these classes for another class.

[0184] Additionally or alternatively, cysteine residues not involved in maintaining the proper conformation of the antibody or antigen-binding fragment may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

[0185] In some embodiments, a substitutional variant comprises a substitution within one or more hypervariable region residues of a parent antibody (*e.g.*, a human antibody). Generally, the resulting variant(s) having improved biological properties relative to the parent antibody from which they are generated are selected for further development.

[0186] A method for generating such substitutional variants involves affinity maturation using phage display. In an example of such a method, several hypervariable region sites (*e.g.*, 6-7 sites) are mutated to generate all possible amino substitutions at each site. Antibody variants thus generated are displayed in a monovalent fashion, *e.g.*, from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.*, binding affinity).

[0187] To identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody or antigen-binding fragment and the antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening, and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0188] In some embodiments, the original glycosylation pattern of a parent antibody is altered. Such alteration(s) may comprise deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

[0189] Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are recognition sequences for enzymatic attachment of the carbohydrate moiety to an asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0190] Addition of glycosylation sites to the antibody may be accomplished by altering the antibody or antigen-bindng fragment's amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0191] Nucleic acid molecules encoding amino acid sequence variants of antibodies or antigen-binding fragments may be prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody or antigen-binding fragment thereof.

[0192] In some embodiments, a modification that increases the serum half-life is used. For example, a salvage receptor binding epitope can be incorporated into a molecule containing disclosed antigen-binding site as described, *e.g.*, in U.S. Pat. No. 5,739,277. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG1, IgG2, IgG3, or IgG4) that is responsible for increasing the in vivo serum half-life of the IgG molecule.

Methods of making antigen-binding sites and molecules containing antigen-binding sites

[0193] The proteins herein above can be made using recombinant DNA technology well known to a skilled person in the art.

[0194] For example, one or more nucleic acid sequences encoding a protein containing the disclosed antigen-binding site can be cloned into one or more expression vectors; the expression vectors can be stably transfected into host cells capable of expressing the gene(s). After transfection, single clones can be isolated for cell bank generation using methods known in the art, such as limited dilution, ELISA, FACS, microscopy, or Clonepix. Clones can be cultured under conditions suitable for bio-reactor scale-up and maintained expression of a protein comprising an antigen-binding site disclosed herein. The protein can be isolated and purified using methods known in the art, such as centrifugation, depth filtration, cell lysis, homogenization, freeze-thawing, affinity purification, gel filtration, ion exchange chromatography, hydrophobic interaction exchange chromatography, and mixed-mode chromatography.

[0195] Accordingly, also provided herein are isolated nucleic acids encoding antigen-binding sites and molecules (e.g., proteins) containing antigen-binding sites, vectors and host cells comprising the nucleic acid, and recombinant techniques for the production.

[0196] In certain embodiments, provided are one or more isolated nucleic acids comprising sequences encoding an immunoglobulin heavy chain and/or immunoglobulin light chain variable region of any antibody disclosed herein, and one or more expression vectors that express the immunoglobulin heavy chain and/or immunoglobulin light chain variable region of any antibody disclosed herein. Also provided are host cells comprising one or more of the foregoing expression vectors and/or isolated nucleic acids.

[0197] In some embodiments, antigen-binding sites and molecules (e.g., proteins) containing antigen-binding sites of the present disclosure may be fused to another agent, *e.g.*, another therapeutic agent. Construction of fusion proteins is within ordinary skill in the art.

Monoclonal Antibodies

[0198] Monoclonal antibodies may be made using the hybridoma method first described by, or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567).

[0199] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively or additionally, lymphocytes may be immunized in vitro. After immunization, lymphocytes are isolated and then fused with a myeloma cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell.

[0200] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium which medium preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells (also referred to as fusion partner). For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the selective culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[0201] Examples of suitable fusion partner include, but are not limited to, myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the unfused parental cells. Examples of suitable myeloma cell lines include, but are not limited to, are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 and derivatives e.g., X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies.

[0202] According to the hybridoma method, culture medium in which hybridoma cells are growing is then assayed for production of monoclonal antibodies directed against the antigen. For example, the binding specificity of monoclonal antibodies produced by hybridoma cells may be determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

[0203] The binding affinity of the monoclonal antibody can, for example, be determined by a Scatchard analysis.

[0204] Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, clones may be subcloned, *e.g.*, by limiting dilution procedures and grown by standard methods. Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal, *e.g.*, by i.p. injection of the cells into mice.

[0205] Monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, affinity chromatography (*e.g.*, using protein A or protein G-Sepharose®) or ion-exchange chromatography, hydroxylapatite chromatography, gel electrophoresis, dialysis, etc.

[0206] DNA encoding the monoclonal antibodies can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Hybridoma cells can serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells.

[0207] In certain embodiments, monoclonal antibodies or antibody fragments are isolated from antibody phage libraries. High affinity (nM range) human antibodies can be produced, *e.g.*, by chain shuffling. Combinatorial infection and *in vivo* recombination may provide strategies for constructing very large phage libraries. These techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[0208] DNA that encodes the antibody may be modified to produce chimeric or fusion antibody polypeptides, for example, by substituting human heavy chain and light chain constant domain (CH and CL) sequences for the homologous murine sequences (*see, e.g.*, U.S. Pat. No. 4,816,567), or by fusing the immunoglobulin coding sequence with all or part of the coding sequence for a non-immunoglobulin polypeptide (heterologous polypeptide).

Human Antibodies and Phage Display Methodology

[0209] Human antibodies can be generated by methods known in the art, including methods described herein. For example, it is possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. *See, e.g.*, U.S. Pat. Nos. 5,545,806, 5,569,825, 5,591,669; 5,545,807; and WO 97/17852.

[0210] Alternatively, phage display technology can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are

cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats. Several sources of V-gene segments can be used for phage display, e.g., from random combinatorial librarues of V genes such as libraries derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following methods described in the art. *See, e.g.*, U.S. Pat. Nos. 5,565,332 and 5,573,905.

[0211] Human antibodies may also be generated by in vitro activated B cells (*see, e.g.*, U.S. Pat. Nos. 5,567,610 and 5,229,275).

[0212] Competition assays for determining whether an antibody binds to the same epitope as, or competes for binding with a disclosed antibody are known in the art. Exemplary competition assays include immunoassays (*e.g.*, ELISA assays, RIA assays), surface plasmon resonance (*e.g.*, BIAcore analysis), bio-layer interferometry, and flow cytometry.

[0213] Typically, a competition assay involves the use of an antigen bound to a solid surface or expressed on a cell surface, a test antibody, and a reference antibody. The reference antibody is labeled and the test antibody is unlabeled. Competitive inhibition is measured by determining the amount of labeled reference antibody bound to the solid surface or cells in the presence of the test antibody. Usually the test antibody is present in excess (*e.g.*, 1x, 5x, 10x, 20x or 100x). Antibodies identified by competition assay (*e.g.*, competing antibodies) include antibodies binding to the same epitope, or similar (*e.g.*, overlapping) epitopes, as the reference antibody, and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur.

[0214] A competition assay can be conducted in both directions to ensure that the presence of the label does not interfere or otherwise inhibit binding. For example, in the first direction the reference antibody is labeled and the test antibody is unlabeled, and in the second direction, the test antibody is labeled and the reference antibody is unlabeled.

[0215] A test antibody competes with the reference antibody for specific binding to the antigen if an excess of one antibody (*e.g.*, 1x, 5x, 10x, 20x or 100x) inhibits binding of the

other antibody, *e.g.*, by at least 50%, 75%, 90%, 95% or 99% as measured in a competitive binding assay.

[0216] Two antibodies may be determined to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies may be determined to bind to overlapping epitopes if only a subset of the amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0217] The antibodies disclosed herein may be further optimized (*e.g.*, affinity-matured) to improve biochemical characteristics including affinity and/or specificity, improve biophysical properties including aggregation, stability, precipitation and/or non-specific interactions, and/or to reduce immunogenicity. Affinity-maturation procedures are within ordinary skill in the art. For example, diversity can be introduced into an immunoglobulin heavy chain and/or an immunoglobulin light chain by DNA shuffling, chain shuffling, CDR shuffling, random mutagenesis and/or site-specific mutagenesis.

[0218] In certain embodiments, isolated human antibodies contain one or more somatic mutations. In these cases, antibodies can be modified to a human germline sequence to optimize the antibody (*e.g.*, by a process referred to as germlining).

[0219] Generally, an optimized antibody has at least the same, or substantially the same, affinity for the antigen as the non-optimized (or parental) antibody from which it was derived. For example, in certain embodiments, an optimized antibody has a higher affinity for the antigen when compared to the parental antibody.

Pharmaceutical compositions

[0220] In certain embodiments, provided molecules containing disclosed antigen-binding sites are incorporated together with one or more pharmaceutically acceptable carriers into a pharmaceutical composition suitable for administration to a subject. As used herein, "pharmaceutically acceptable carrier" refers to any of a variety of solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include, but are not limited to, water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof.

[0221] In some embodiments, pharmaceutical compositions comprise one or more tonicity agents or stabilizers. Non-limiting examples of such tonicity agents or stabilizers include sugars (e.g., sucrose), polyalcohols (e.g., mannitol or sorbitol), and sodium chloride.

[0222] In some embodiments, pharmaceutical compositions comprise one or more bulking agents and/or lyoprotectants (*e.g.,* mannitol or glycine), buffers (e.g., phosphate, acetate, or histidine buffers), surfactants (*e.g.,* polysorbates), antioxidants (*e.g.*, methionine), and/or metal ions or chelating agents (*e.g.*, ethylenediaminetetraacetic acid (EDTA)).

[0223] In some embodiments, pharmaceutical compositions comprise one or more auxiliary substances such as wetting or emulsifying agents, preservatives (*e.g.,* benzyl alcohol) or buffers, which may enhance the shelf life and/or effectiveness of immunoconjugates disclosed herein.

[0224] Pharmaceutical compositions may be provided in any of a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. Suitability of certain forms may depend on the intended mode of administration and therapeutic application.

[0225] In some embodiments, pharmaceutical compositions are in the form of injectable or infusible solutions.

[0226] Pharmaceutical compositions are typically sterile and stable under conditions of manufacture, transport, and storage. Pharmaceutical compositions may be formulated as, for example, a solution, microemulsion, dispersion, liposome, or other ordered structure. In some embodiments, a pharmaceutical composition is formulated as a structure particularly suitable for high drug concentration. For example, sterile injectable solutions can be prepared by incorporating a therapeutic agent (*e.g.,* immunoconjugate) in a desired amount in an appropriate solvent with one or a combination of ingredients enumerated herein, optionally followed by sterilization (*e.g.*, filter sterilization). Generally, dispersions may be prepared by incorporating an immunoconjugate into a sterile vehicle that contains a basic dispersion medium and other ingredient(s) such as those additional ingredients mentioned herein. In the case of sterile powders for the preparation of sterile injectable solutions, examples of preparation methods include vacuum drying and freeze-drying to yield a powder of the immunoconjugate and any additional desired ingredient(s), *e.g.*, from a previously sterile-filtered solution thereof.

[0227] Proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by maintaining certain particle sizes (*e.g.,* in the case of dispersions), and/or by using surfactants. Prolonged absorption of injectable compositions can be brought about, *e.g.,* by including in the composition an agent that delays absorption (for example, monostearate salts and/or gelatin).

Methods of Treatment

[0228] The present application provides methods for treating a disease or disorder using a molecule (*e.g.*, protein, such as antibody or antigen-binding fragment thereof) or pharmaceutical composition described herein. Methods of treatment disclosed herein generally comprise a step of administering a therapeutically effective amount of a disclosed molecule containing antigen-binding site or a pharmaceutical composition thereof to a subject (*e.g.,* a human subject) in need thereof.

[0229] In some embodiments, the disease or disorder is a mast cell associated disease or disorder. In some embodiments, the mast cell associated disease or disorder is related to undesirable mast cell activity, e.g., excessive proliferation of mast cells. Non-limiting examples of mast cell associated diseases or disorders include upper airway diseases such as allergic rhinitis and sinusitis, foreign body aspiration, glottic stenosis, tracheal stenosis, laryngotracheomalacia, vascular rings, chronic obstructive pulmonary disease (COPD), and congestive heart failure, eosinophilic bronchitis, polychondritis, sarcoidosis, papillomatosis, arthritis (e.g., rheumatoid arthritis), Wegener's granulomatosis, primary biliary cirrhosis, an allergy that is responsive to oral immunotherapy, secondary sclerosing cholangitis due to mast cell cholangiopathy, pruritus, itching caused by or associated with chronic kidney disease associated pruritis or primary biliary cirrohosis, bronchiolitis obliterans syndrome after lung transplantation or hematopoietic stem cell transplantation, inflammatory bowel syndrome, functional dyspepsia, bronchopulmonary dysplasia in infants, cerebral ischemia, seizures, pneumonia caused by influenza, sepsis, scleroderma, cluster headaches, cardiac fibrosis, primary sclerosing cholangitis (PSC), Morbihan disease, aspirin intolerant asthma, vulvodynia, chronic pelvic pain syndrome (prostatitis), and cancer (e.g., lung cancer).

[0230] In some embodiments, the disease or disorder is an autoimmune disease. In some embodiments, the disease or disorder is an allergy. In some embodiments, the disease or disorder is an inflammation. In some embodiments, the disease or disorder is pruritus. In some embodiments, the disease or disorder is a cancer.

[0231] Therapeutically effective amounts may be administered via a single dose or via multiple doses (*e.g.,* at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten doses). When administered via multiple doses, any of a variety of suitable therapeutic regimens may be used, including administration at regular intervals (*e.g.,* once every other day, once every three days, once every four days,

once every five days, thrice weekly, twice weekly, once a week, once every two weeks, once every three weeks, etc.).

[0232] The dosage regimen (*e.g.*, amounts of each therapeutic, relative timing of therapies, etc.) that is effective in methods of treatment may depend on the severity of the disease or condition and the weight and general state of the subject. For example, the therapeutically effective amount of a particular composition comprising a therapeutic agent applied to mammals (*e.g.*, humans) can be determined by the ordinarily-skilled artisan with consideration of individual differences in age, weight, and the condition of the mammal. Therapeutically effective and/or optimal amounts can also be determined empirically by those of skill in the art.

[0233] The molecule containing antigen-binding site or pharmaceutical composition thereof may be administered by any of a variety of suitable routes, including, but not limited to, systemic routes such as parenteral (*e.g.*, intravenous or subcutaneous) or enteral routes.

List of Exemplary Embodiments

[0234] The invention is further described by the following non-limiting exemplary embodiments:

Enbodiment 1. An antigen-binding site that is capable of binding to FCER1A, FCER1B, and/or FCER1G.

Enbodiment 2. The antigen-binding site of embodiment 1, wherein the antigen-binding site is capable of binding to FCER1A.

Enbodiment 3. The antigen-binding site of embodiment 1, wherein the antigen-binding site is capable of binding to FCER1B.

Enbodiment 4. The antigen-binding site of embodiment 1, wherein the antigen-binding site is capable of binding to FCER1G.

Enbodiment 5. An antigen-binding site that is capable of binding to c-Kit.

Enbodiment 6. An antigen-binding site that is capable of binding to siglec-6.

Enbodiment 7. An antigen-binding site that is capable of binding to siglec-8.

Enbodiment 8. The antigen-binding site of any one of embodiments 1-7, wherein the antigen-binding site is present as a single-chain fragment variable (scFv).

Enbodiment 9. The antigen-binding site of any one of embodiments 1-7, wherein the antigen-binding site is present as an antibody mimetic (e.g., an Affibody, an Affilin, an Affimer, an afftin, an Alphabody, an Anticalin, an avimer, a Centyrin, a DARPin, a Fynomer, a monobody, or a nanoCLAMP).

Enbodiment 10. The antigen-binding site of any one of embodiments 5 and 8-9, wherein the antigen-binding site comprises

(a) the complementarity-determining region 1 (CDR1), CDR2, and/or CDR3 of a heavy chain (HC) set forth in any one of SEQ ID NO: 39, 49, 51, 54, 56, or 58; and/or

(b) the CDR1, CDR2, and/or CDR3 of a light chain (LC) set forth in any one of SEQ ID NO: 44, 50, 86, or 88.

Enbodiment 11. The antigen-binding site of any one of embodiments 5 and 8-10, wherein the antigen-binding site comprises

(a) a heavy chain variable domain (VH) comprising complementarity-determining regions:

CDR-H1 comprising the amino acid sequence: SYNMH (SEQ ID NO: 41),

CDR-H2 comprising the amino acid sequence: VIYSGNGDTSYNQKFKG (SEQ ID NO: 42) or IYSGNGDTSYNQKFKGK (SEQ ID NO: 33), and

CDR-H3 comprising the amino acid sequence: ERDTRFGN (SEQ ID NO: 43); and/or

(b) a light chain variable domain (VL) comprising complementarity-determining regions:

CDR-L1 comprising the amino acid sequence: RASESVDIYGNSFMH (SEQ ID NO: 46);

CDR-L2 comprising the amino acid sequence: LASNLES (SEQ ID NO: 47); and

CDR-L3 comprising the amino acid sequence: QQNNEDPYT (SEQ ID NO: 48).

Enbodiment 12. The antigen-binding site of any one of embodiments 5 and 8-11, wherein the antigen-binding site comprises

(a) a VH comprising an amnio acid sequence having at least 85% identity to the VH of a HC set forth in SEQ ID NO: 39; and/or

a VL comprising an amnio acid sequence having at least 85% identity to the VL of a LC set forth in SEQ ID NO: 44; or

(b) a VH comprising an amnio acid sequence having at least 85% identity to the VH of a HC set forth in SEQ ID NO: 49; and/or

a VL comprising an amnio acid sequence having at least 85% identity to the VL of a LC set forth in SEQ ID NO: 50; or

(c) a VH comprising an amnio acid sequence having at least 85% identity to the VH of a HC set forth in any one of SEQ ID NOs: 51, 54, 56, and 58; and/or

a VL comprising an amnio acid sequence having at least 85% identity to the VL of a LC set forth in SEQ ID NO: 86 or 88.

Enbodiment 13. The antigen-binding site of any one of embodiments 5 and 8-12, wherein the antigen-binding site comprises

(a) a HC comprising CDR-H1, CDR-H2, and CDR-H3 of a HC set forth in SEQ ID NO: 39, and having at least 85% amino acid sequence identity to SEQ ID NO: 39; and/or
a LC comprising CDR-L1, CDR-L2, and CDR-L3 of a LC set forth in SEQ ID NO: 44, and having at least 85% amino acid sequence identity to SEQ ID NO: 44; or

(b) a HC comprising CDR-H1, CDR-H2, and CDR-H3 of a HC set forth in SEQ ID NO: 49, and having at least 85% amino acid sequence identity to SEQ ID NO: 49; and/or
a LC comprising CDR-L1, CDR-L2, and CDR-L3 of a LC set forth in SEQ ID NO: 50, and having at least 85% amino acid sequence identity to SEQ ID NO: 50; or

(c) a HC comprising CDR-H1, CDR-H2, and CDR-H3 of a HC set forth in any one of SEQ ID NOs: 51, 54, 56, and 58, and having at least 85% amino acid sequence identity thereof; and/or
a LC comprising CDR-L1, CDR-L2, and CDR-L3 of a LC set forth in SEQ ID NO: 86 or 88, and having at least 85% amino acid sequence identity thereof.

Enbodiment 14. The antigen-binding site of embodiment 12 or 13, wherein the sequence identity is at least 90%, 95%, 96%, 97%, 98%, or 99%.

Enbodiment 15. The antigen-binding site of any one of embodiments 5 and 8-14, wherein the antigen-binding site comprises

(a) the VH of a HC set forth in SEQ ID NO: 39; and/or the VL of a LC set forth in SEQ ID NO: 44; or

(b) the VH of a HC set forth in SEQ ID NO: 49; and/or the VL of a LC set forth in SEQ ID NO: 50; or

(c) the VH of a HC set forth in any one of SEQ ID NOs: 51, 54, 56, and 58; and/or the VL of a LC set forth in SEQ ID NO: 86 or 88.

Enbodiment 16. The antigen-binding site of any one of embodiments 6 and 8-9, wherein the antigen-binding site comprises

(a) the CDR1, CDR2, and/or CDR3 of a HC set forth in any one of SEQ ID NO: 1, 17, 19, 22, 24, 26, or 28; and/or

(b) the CDR1, CDR2, and/or CDR3 of a LC set forth in any one of SEQ ID NO: 9, 18, 30, 35, or 37.

Enbodiment 17. The antigen-binding site of any one of embodiments 6, 8-9, and 16, wherein the antigen-binding site comprises

(a) a VH comprising complementarity-determining regions:

CDR-H1 comprising the amino acid sequence: SYWMH (SEQ ID NO: 3),

CDR-H2 comprising the amino acid sequence: EIYPTNGGTTYNEKFKR (SEQ ID NO: 4), and

CDR-H3 comprising the amino acid sequence: EDFYAMDY (SEQ ID NO: 5); and/or

(b) a VL comprising complementarity-determining regions:

CDR-L1 comprising the amino acid sequence: KSSQSLLDSDGKTCLN (SEQ ID NO: 11);

CDR-L2 comprising the amino acid sequence: LVSKLDS (SEQ ID NO: 12); and

CDR-L3 comprising the amino acid sequence: WQGTHFPYT (SEQ ID NO: 13).

Enbodiment 18. The antigen-binding site of any one of embodiments 6, 8-9, and 16, wherein the antigen-binding site comprises

(a) a VH comprising complementarity-determining regions:

CDR-H1 comprising the amino acid sequence: GYAFTSYWMH (SEQ ID NO: 6),

CDR-H2 comprising the amino acid sequence: EIYPTNGGTT (SEQ ID NO: 7), and

CDR-H3 comprising the amino acid sequence: EDFYAMDY (SEQ ID NO: 8); and/or

(b) a VL comprising complementarity-determining regions:

CDR-L1 comprising the amino acid sequence: KSSQSLLDSDGKTCLN (SEQ ID NO: 14);

CDR-L2 comprising the amino acid sequence: LVSKLDS (SEQ ID NO: 15); and

CDR-L3 comprising the amino acid sequence: WQGTHFPYT (SEQ ID NO: 16).

Enbodiment 19. The antigen-binding site of any one of embodiments 6, 8-9, and 16-18, wherein the antigen-binding site comprises

(a) a VH comprising an amnio acid sequence having at least 85% identity to the VH of a HC set forth in SEQ ID NO: 1; and/or

a VL comprising an amnio acid sequence having at least 85% identity to the VL of a LC set forth in SEQ ID NO: 9; or

(b) a VH comprising an amnio acid sequence having at least 85% identity to the VH of a HC set forth in SEQ ID NO: 17; and/or

a VL comprising an amnio acid sequence having at least 85% identity to the VL of a LC set forth in SEQ ID NO: 18; or

(c) a VH comprising an amnio acid sequence having at least 85% identity to the VH of a HC set forth in any one of SEQ ID NOs: 19, 22, 24, 26, and 28; and/or

a VL comprising an amnio acid sequence having at least 85% identity to the VL of a LC set forth in any one of SEQ ID NOs: 30, 35, and 37.

Enbodiment 20. The antigen-binding site of any one of embodiments 6, 8-9, and 16-19, wherein the antigen-binding site comprises

(a) a HC comprising CDR-H1, CDR-H2, and CDR-H3 of a HC set forth in SEQ ID NO: 1, and having at least 85% amino acid sequence identity to SEQ ID NO: 1; and/or
a LC comprising CDR-L1, CDR-L2, and CDR-L3 of a LC set forth in SEQ ID NO: 9, and having at least 85% amino acid sequence identity to SEQ ID NO: 9; or

(b) a HC comprising CDR-H1, CDR-H2, and CDR-H3 of a HC set forth in SEQ ID NO: 17, and having at least 85% amino acid sequence identity to SEQ ID NO: 17; and/or
a LC comprising CDR-L1, CDR-L2, and CDR-L3 of a LC set forth in SEQ ID NO: 18, and having at least 85% amino acid sequence identity to SEQ ID NO: 18; or

(c) a HC comprising CDR-H1, CDR-H2, and CDR-H3 of a HC set forth in any one of SEQ ID NOs: 19, 22, 24, 26, and 28, and having at least 85% amino acid sequence identity thereof; and/or
a LC comprising CDR-L1, CDR-L2, and CDR-L3 of a LC set forth in any one of SEQ ID NOs: 30, 35, and 37, and having at least 85% amino acid sequence identity thereof.

Enbodiment 21. The antigen-binding site of embodiment 19 or 20, wherein the sequence identity is at least 90%, 95%, 96%, 97%, 98%, or 99%.

Enbodiment 22. The antigen-binding site of any one of embodiments 6, 8-9, and 16-21, wherein the antigen-binding site comprises

(a) the VH of a HC set forth in SEQ ID NO: 1; and/or the VL of a LC set forth in SEQ ID NO: 9; or

(b) the VH of a HC set forth in SEQ ID NO: 17; and/or the VL of a LC set forth in SEQ ID NO: 18; or

(c) the VH of a HC set forth in any one of SEQ ID NOs: 19, 22, 24, 26, and 28; and/or the VL of a LC set forth in any one of SEQ ID NOs: 30, 35, and 37.

Enbodiment 23. The antigen-binding site of any one of embodiments 6-9, wherein the antigen-binding site comprises

(a) the CDR1, CDR2, and/or CDR3 of a VH set forth in SEQ ID NO: 121; and/or

(b) the CDR1, CDR2, and/or CDR3 of a LC set forth in SEQ ID NO: 126.

Enbodiment 24. The antigen-binding site of any one of embodiments 6-9 and 23, wherein the antigen-binding site comprises

(a) a VH comprising complementarity-determining regions:

CDR-H1 comprising the amino acid sequence: DYGMH (SEQ ID NO: 122),

CDR-H2 comprising the amino acid sequence: GISWNSGSIGYADSVKG (SEQ ID NO: 123), and

CDR-H3 comprising the amino acid sequence: GGQTIDI (SEQ ID NO: 124); and/or

(b) a VL comprising complementarity-determining regions:

CDR-L1 comprising the amino acid sequence: RASQSISSYLN (SEQ ID NO: 127);

CDR-L2 comprising the amino acid sequence: AASSLQS (SEQ ID NO: 128); and

CDR-L3 comprising the amino acid sequence: QQSYSTPFT (SEQ ID NO: 129).

Enbodiment 25. The antigen-binding site of any one of embodiments 6-9 and 23-24, wherein the antigen-binding site comprises

a VH comprising an amnio acid sequence having at least 85% to SEQ ID NO: 121; and/or

a VL comprising an amnio acid sequence having at least 85% to SEQ ID NO: 126.

Enbodiment 26. The antigen-binding site of any one of embodiments 6-9 and 23-25, wherein the antigen-binding site comprises

a HC comprising CDR-H1, CDR-H2, and CDR-H3 of a HC set forth in SEQ ID NO: 120, and having at least 85% amino acid sequence identity to SEQ ID NO: 120; and/or

a LC comprising CDR-L1, CDR-L2, and CDR-L3 of a LC set forth in SEQ ID NO: 125, and having at least 85% amino acid sequence identity to SEQ ID NO: 125.

Enbodiment 27. The antigen-binding site of embodiment 25 or 26, wherein the sequence identity is at least 90%, 95%, 96%, 97%, 98%, or 99%.

Enbodiment 28. The antigen-binding site of any one of embodiments 6-9 and 23-27, wherein the antigen-binding site comprises a VH comprising an amnio acid sequence set forth in SEQ ID NO: 121; and/or a VL comprising an amnio acid sequence set forth in SEQ ID NO: 126.

Enbodiment 29. An antibody or antigen-binding fragment thereof comprising an antigen-binding site of any one of embodiments 1-4.

Enbodiment 30. An antibody or antigen-binding fragment thereof comprising an antigen-binding site of any one of embodiments 5 and 10-15.

Enbodiment 31. The antibody or antigen-binding fragment thereof of embodiment 30, capable of blocking c-kit signaling.

Enbodiment 32. An antibody or antigen-binding fragment thereof comprising an antigen-binding site of any one of embodiments 6 and 16-22.

Enbodiment 33. The antibody or antigen-binding fragment thereof of embodiment 32, capable of agonizing siglec-6.

Enbodiment 34. An antibody or antigen-binding fragment thereof comprising an antigen-binding site of embodiment 7.

Enbodiment 35. An antibody or antigen-binding fragment thereof comprising an antigen-binding site of any one of embodiments 23-28.

Enbodiment 36. The antibody or antigen-binding fragment thereof of any one of embodiments 29-35, wherein the antibody is a humanized antibody.

Enbodiment 37. The antibody or antigen-binding fragment thereof of any one of embodiments 29-35, wherein the antibody is a human antibody.

Enbodiment 38. The antibody or antigen-binding fragment thereof of any one of embodiments 29-35, wherein the antibody is a multispecific antibody.

Enbodiment 39. The antibody or antigen-binding fragment thereof of any one of embodiments 29-35, wherein the antibody is a bispecific antibody.

Enbodiment 40. The antibody or antigen binding fragment thereof of any one of embodiments 29-35, wherein the antigen binding fragment is a Fab, a F(ab')2, a Fab', an scFv, an Fv fragment, a Fd fragment, or a diabody.

Enbodiment 41. The antibody or antigen binding fragment thereof of any one of embodiments 29-35, wherein the antibody or antigen binding fragment thereof comprises an antibody heavy chain constant region.

Enbodiment 42. The antibody or antigen binding fragment thereof of embodiment 41, wherein the antibody heavy chain constant region is a human IgG heavy chain constant region.

Enbodiment 43. The antibody or antigen binding fragment thereof of any one of embodiments 29-42, wherein the antibody or antigen binding fragment thereof has reduced or null effector function.

Enbodiment 44. The antibody or antigen binding fragment thereof of any one of embodiments 29-43, wherein the antibody or antigen binding fragment thereof is afucosylated.

Enbodiment 45. The antibody or antigen binding fragment thereof of embodiment 44, wherein the antibody or antigen binding fragment thereof is at least about 75% afucosylated.

Enbodiment 46. The antibody or antigen-binding fragment thereof of embodiment 44 or 45, wherein the antibody or antigen binding fragment thereof is capable of depleting mast cell.

Enbodiment 47. A protein comprising an antigen-binding site of any one of embodiments 1-4.

Enbodiment 48. A protein comprising an antigen-binding site of any one of embodiments 5 and 10-15.

Enbodiment 49. A protein comprising an antigen-binding site of any one of embodiments 6 and 16-22.

Enbodiment 50. A protein comprising an antigen-binding site of embodiment 7.

Enbodiment 51. A protein comprising an antigen-binding site of any one of embodiments 23-28

Enbodiment 52. A protein comprising two or more antigen-binding sites selected from: (i) an antigen-binding site of any one of embodiments 1-4; (ii) an antigen-binding site of any one of embodiments 5 and 10-15; (iii) an antigen-binding site of any one of embodiments 6 and 16-22; (iv) an antigen-binding site of embodiment 7; and (v) an antigen-binding site of any one of embodiments 23-28.

Enbodiment 53. A protein comprising two antigen-binding sites selected from: (i) an antigen-binding site of any one of embodiments 1-4; (ii) an antigen-binding site of any one of embodiments 5 and 10-15; (iii) an antigen-binding site of any one of embodiments 6 and 16-22; (iv) an antigen-binding site of embodiment 7; and (v) an antigen-binding site of any one of embodiments 23-28.

Enbodiment 54. A protein comprising

(i) an antigen-binding site of any one of embodiments 5 and 10-15; and

(ii) an antigen-binding site selected from: (a) an antigen-binding site of embodiment 2, (b) an antigen-binding site of embodiment 3, or (c) an antigen-binding site of one of embodiments 6 and 16-22.

Enbodiment 55. The protein of any one of embodiments 47-54, further comprising an antibody heavy chain constant region.

Enbodiment 56. The protein of embodiment 55, wherein the antibody heavy chain constant region is a human IgG heavy chain constant region.

Enbodiment 57. The protein of any one of embodiments 47-56, wherein the protein has reduced or null effector function.

Enbodiment 58. The protein of any one of embodiments 47-57, wherein the protein is afucosylated.

Enbodiment 59. The protein of embodiment 58, wherein the protein is at least about 75% afucosylated.

Enbodiment 60. The protein of embodiment 58 or 59, wherein the protein is capable of depleting mast cell.

Enbodiment 61. An isolated nucleic acid encoding an antigen-binding site of any one of embodiments 1-28, an antibody or antigen-binding fragment thereof of any one of embodiments 29-46, or a protein of any one of embodiments 47-60.

Enbodiment 62. An expression vector comprising the isolated nucleic acid of embodiment 61.

Enbodiment 63. A host cell comprising the isolated nucleic acid molecule of embodiment 61 or the expression vector of embodiment 62.

Enbodiment 64. A pharmaceutical composition comprising an antigen-binding site of any one of embodiments 1-28, an antibody or antigen-binding fragment thereof of any one of embodiments 29-46, or a protein of any one of embodiments 47-60, and a pharmaceutically acceptable carrier.

Enbodiment 65. A method of treating a disease or disorder in a subject in need thereof, comprising administering to the subject an effective amount of an antigen-binding site of any one of embodiments 1-28, an antibody or antigen-binding fragment thereof of any one of embodiments 29-46, a protein of any one of embodiments 47-60, or a pharmaceutical composition of embodiment 64.

Enbodiment 66. The method of embodiment 65, wherein the disease or disorder is a mast cell associated disease or disorder.

Enbodiment 67. The method of embodiment 65, wherein the disease or disorder is an autoimmune disease.

Enbodiment 68. The method of embodiment 65, wherein the disease or disorder is an allergy.

Enbodiment 69. The method of embodiment 65, wherein the disease or disorder is an inflammation.

Enbodiment 70. The method of embodiment 65, wherein the disease or disorder is pruritus.

Enbodiment 71. The method of embodiment 65, wherein the disease or disorder is a cancer.

Enbodiment 72. The antibody or antigen-binding fragment thereof of embodiment 12 or 13 that comprises an amino acid sequence selected from any one of SEQ ID NOs: 1, 9, 17-19, 22, 24, 26, 28, 30, 35, 37, 120, 121, 125, and 126, or a fragment thereof.

Enbodiment 73. The antibody or antigen-binding fragment thereof of embodiment 12 or 13 that comprises at least one amino acid sequence selected from any one of SEQ ID NOs: 1, 9, 17-19, 22, 24, 26, 28, 30, 35, 37, 120, 121, 125, and 126, or a fragment thereof.

Enbodiment 74. The antibody or antigen-binding fragment thereof of embodiment 12 or 13 that comprises at least one CDR sequence found in sequences selected from any one of SEQ ID NOs: 1, 9, 17-19, 22, 24, 26, 28, 30, 35, 37, 120, 121, 125, and 126.

Enbodiment 75. The antibody or antigen-binding fragment thereof of embodiment 12 or 13 that comprises at least two CDR sequences found in sequences selected from any one of SEQ ID NOs: 1, 9, 17-19, 22, 24, 26, 28, 30, 35, 37, 120, 121, 125, and 126.

Enbodiment 76. The antibody or antigen-binding fragment thereof of embodiment 12 or 13 that comprises at least three CDR sequences found in sequences selected from any one of SEQ ID NOs: 1, 9, 17-19, 22, 24, 26, 28, 30, 35, 37, 120, 121, 125, and 126.

Enbodiment 77. The antibody or antigen-binding fragment thereof of embodiment 11 that comprises an amino acid sequence selected from any one of SEQ ID NOs: 39, 44, 49-51, 54, 56, 58, 86, and 88, or a fragment thereof.

Enbodiment 78. The antibody or antigen-binding fragment thereof of embodiment 11 that comprises at least one amino acid sequence selected from any one of SEQ ID NOs: 39, 44, 49-51, 54, 56, 58, 86, and 88, or a fragment thereof.

Enbodiment 79. The antibody or antigen-binding fragment thereof of embodiment 11 that comprises at least two amino acid sequences selected from any one of SEQ ID NOs: 39, 44, 49-51, 54, 56, 58, 86, and 88, or a fragment thereof.

Enbodiment 80. The antibody or antigen-binding fragment thereof of embodiment 11 that comprises at least one CDR found in a sequence selected from any one of SEQ ID NOs: 39, 44, 49-51, 54, 56, 58, 86, and 88, or a fragment thereof.

Enbodiment 81. The antibody or antigen-binding fragment thereof of embodiment 11 that comprises at least two CDRs found in sequences selected from any one of SEQ ID NOs: 39, 44, 49-51, 54, 56, 58, 86, and 88, or a fragment thereof.

Enbodiment 82. The antibody or antigen-binding fragment thereof of embodiment 11 that comprises at least three CDRs found in sequences selected from any one of SEQ ID NOs: 39, 44, 49-51, 54, 56, 58, 86, and 88, or a fragment thereof.

Enbodiment 83. An antibody or antigen-binding fragment that comprises an amino acid sequence selected any one of SEQ ID NOs: 1, 9, 17-19, 22, 24, 26, 28, 30, 35, 37, 39, 44, 49-51, 54, 56, 58, 86, 88, 120, 121, 125, and 126, or a fragment thereof.

EXAMPLES

Example 1. Antibody production

[0235] FCER1, c-Kit, and siglec (e.g., siglec-6 and siglec 8) targeting huaman antibodies are produced by methods known in the art.

[0236] In some embodiments, transgenic mice that are incapable of expressing functional endogenous immunoglobulins but express human immunoglobulin genes are used. In particular, the human heavy and light chain immunoglobulin gene complexes are introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region are introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes are rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of an antigen (e.g., FCER1, c-Kit, or sigle). Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation.

[0237] In some other embodiments, FCER1, c-Kit, and siglec (e.g., siglec-6 and siglec 8) targeting antibodies are produced by screening yeast display libraries.

[0238] Further, c-Kit/siglec-6 bispecific antibodies are produced by methods known in the art. In some embodiments, c-Kit/siglec-6 bispecific antibodies are in CrossMab format.

Example 2. Binding of antibodies toward c-Kit

[0239] Target binding of c-Kit antibodies to c-Kit-expressing cells was assessed by flow cytometry. Cultured M-07e cells were resuspended in FACS Blocking Buffer (PBS + 10% normal human serum) for 30 minutes. Following washing, cells were incubated with c-Kit antiboties in FACS Buffer (PBS + 0.5% bovine serum albumin + 0.09% sodium azide) and incubated at 4 °C for 30 minutes, washed and incubated with PE-labeled anti-human secondary antibody. Cells were incubated at 4 °C for 30 minutes in the dark. Following washing, cytofluorimetric analysis was conducted using a BD FACS Symphony A3 (BD

Biosciences). Cells were sorted by scatter properties and separated for single cell analysis using side scatter width vs side scatter area. Antibody binding to cells was quantitated by measuring fluorescent emissions of excitation wavelength of 561nm using a 586/15 nm filter. Data analysis was performed using FlowJo Software and summarized in **Table 2**. All tested c-Kit antiboties were capable of binding to cells expressing c-Kit.

Table 2. Binding of c-Kit antibodies to cell surface expressed c-Kit on M-07e cells

Antibody	**SEQ ID NO**	**Percent Positive for c-Kit on M-07e cells**	**Measuring Fluorescent Intensity (MFI)**
hSR1-1	51 and 86	99.5	5021
hSR1-2	54 and 86	99.7	5199
hSR1-3	56 and 86	99.8	5187
hSR1-4	58 and 86	99.8	4962
hSR1-5	51 and 88	99.5	4029
hSR1-6	54 and 88	99.4	3591
chSR1	49 and 50	99.7	5344
hIgG1 control		2.3	85

[0240] Further, binding affinities of c-Kit antibodies to human c-Kit and cynomolgus c-Kit were measured by biolayer interferometry (BLA) using an Octect instrument. Briefly, capture sensors were loaded with test antibodies with 3-fold dilutions. The association and dissociation rates to were determined and used to calculate the equilibrium dissociation constant K_D (**Table 3**). Affinities to c-Kit measured by K_D values were observed in the subnanomolar range for tested humanized c-Kit antibodies (hSR-1-1 to hSR1-6) and in the single digit nanomolar range for tested chimeric c-Kit antibody (chSR1).

Table 3. Binding affinities for human and cynonolgus c-Kit of determined by BLI

Antibody	**Affinity (KD)**	
	Human c-Kit	**Cynomoguls c-Kit**
hSR1-1	1.99E-10	2.320E-10
hSR1-2	2.45E-10	3.885E-10
hSR1-3	2.56E-10	3.323E-10
hSR1-4	3.86E-10	5.633E-10
hSR1-5	2.56E-10	4.045E-10
hSR1-6	2.47E-10	2.959E-10
chSR1	1.89E-09	3.338E-09

Example 3. Cross-reactivity non-specific binding of c-Kit antibodies analyzed by ELISA

[0241] An ELISA-based assay was employed to assess the cross-reactivity and non-specific binding of c-Kit antibodies toward human c-Kit, cynomongus c-Kit, and c-Kit family members.

[0242] His-tagged human c-Kit, His-tagged cynomolgus c-Kit, and His-tagged PDGFRa, PDGFRb, CSF1R, and FLT3 extracellular domains were immobilized on 96 well ELISA plates. Plates were washed with PBST (Phosphate buffered saline + 0.05% Tween 20) and blocked for 1 hour with 1% BSA in PBS. Following washing with PBST, mAbs were added to the coated plate and incubated for 2 hours at room temperature. Plates were washed and incubated with HRP-conjugated goat anti-human IgG secondary antibody for 1 hour at room temperature. Following washing, antibody binding was detected colorimetrically by adding 3,3',5,5'-tetramethylbenzidine (TMB) and incubated at room temperature for 10-20 minutes before adding 2.5N sulfuric acid stopping solution. Absorbance at 450nm was measured on a plate reader (PerkinElmer). Data was analyzed using GraphPad Prism.

[0243] Results for exemplary c-Kit antibodies are depicted in **Table 4**. The c-Kit anibodies were capable of binding to both human and cynomolgus c-Kit but did not cross react with c-Kit family members PDGFRa, PDGFRb, CSF1R, or FLT3.

Table 4. Cross-reactivity of humanized and chimeric c-Kit antibodies

Antibody	**Cross Reactivity**		
	Human c-Kit	**Cyno c-Kit**	**c-Kit Family members**
hSR1-1	+	+	-
hSR1-2	+	+	-
hSR1-3	+	+	-
hSR1-4	+	+	-
hSR1-5	+	+	-
hSR1-6	+	+	-
chSR1	+	+	-

Example 4. Binding of antibodies toward siglec-6

[0244] Target binding of siglec-6 antibodies to siglec-6-expressing cells was assessed by flow cytometry. Cultured HMC1.2 cells were resuspended in FACS Blocking Buffer (PBS + 10% normal human serum) for 30 minutes. Following washing, cells were incubated with siglec-6 antibodies in FACS Buffer (PBS + 0.5% bovine serum albumin + 0.09% sodium azide) and incubated at 4 °C for 30 minutes, washed and incubated with PE-labeled anti-human or anti-mouse secondary antibodies. Cells were incubated at 4°C for 30 minutes in

the dark. Following washing, cytofluorimetric analysis was conducted using a BD FACS Symphony A3 (BD Biosciences). Cells were sorted by scatter properties and separated for single cell analysis using side scatter width vs side scatter area. Antibody binding to cells was quantitated by measuring fluorescent emissions of excitation wavelength of 561nm using a 586/15 nm filter. Data analysis was performed using FlowJo Software and summarized in **Table 5**. All tested siglec-6 antiboties were capable of binding to cells expressing siglec-6.

Table 5. Binding of humanized and chimeric siglec-6 antibodies to cell surface expressed siglec-6 on HMC1.2 cells

Antibody	**SEQ ID NO**	**Percent Positive for Siglec-6 on HMC1.2 cells**	**MFI**
hRND-2	22 and 37	99.9	3134
hRND-3	24 and 37	99.9	3695
hRND-4	26 and 37	100.0	3203
hRND-5	28 and 37	99.9	1942
chRND	17 and 37	100.0	3608
mRND	1 and 9	99.9	1974
hIgG1 control		0.1	212

[0245] Further, binding affinities of siglec-6 antibodies to human siglec-6 were measured by BLA using an Octect instrument. Briefly, capture sensors were loaded with test antibodies with 3-fold dilutions. The association and dissociation rates to were determined and used to calculate the equilibrium dissociation constant K_D (**Table 6**). Affinities to siglec-6 measured by K_D values were observed in the subnanomolar range for tested humanized and chimeric siglec-6 antibodies.

Table 6. Binding affinities for human siglect-6 of determined by BLI

Antibody	**Affinity (KD)**
	Human siglec-6
hRND-2	1.70E-10
hRND-3	1.37E-10
hRND-4	3.15E-10
hRND-5	1.66E-10
chRND	1.01E-10

Example 5. Cross-reactivity non-specific binding of siglec-6 antibodies analyzed by ELISA

[0246] An ELISA-based assay was employed to assess the cross-reactivity and non-specific binding of siglec-6 antibodies toward human siglec-6, cynomongus siglec-6, and siglec-6 family members siglec 1-5, 7-12, 14, and 15.

[0247] Cultured HEK293 cells expressing human siglec-6, cynomolgus siglec-6, or siglec 6 family members were resuspended in FACS Blocking Buffer (PBS + 10% normal human serum) for 30 minutes. Following washing, cells were incubated with siglec-6 antibodies in FACS Buffer (PBS + 0.5% bovine serum albumin + 0.09% sodium azide) and incubated at 4 °C for 30 minutes, washed and incubated with PE-labeled anti-human secondary antibody. Cells were incubated at 4 °C for 30 minutes in the dark. Following washing, cytofluorimetric analysis was conducted using a BD FACS Symphony A3 (BD Biosciences). Cells were sorted by scatter properties and separated for single cell analysis using side scatter width vs side scatter area. Antibody binding to cells was quantitated by measuring fluorescent emissions of excitation wavelength of 561 nm using a 586/15 nm filter. Data analysis was performed using FlowJo Software.

[0248] Results for exemplary siglec-6 antibodies are depicted in **Table 7**. The siglec-6 anibodies were capable of binding to both human and cynomolgus siglec-6but did not cross react with siglec-6 family members siglec 1-5, 7-12, 14, or 15.

Table 7. Cross-reactivity of humanized and chimeric siglec-6 antibodies

Antibody	**Cross Reactivity**		
	Human siglec-6	**Cyno siglec-6**	**Siglec-6 Family members**
hRND-2	+	+	-
hRND-3	+	+	-
hRND-4	+	+	-
hRND-5	+	+	-
hRND-6	+	+	-
chRND	+	+	-

Example 6. Inhibition of cell proliferation by c-Kit antibodies

[0249] The ability of c-Kit antibodies to inhibit stem cell factor (SCF)-dependent cell proliferation was assessed.

[0250] M-07e cells were grown according to distributor's recommendation. Cells were starved of SCF overnight (16 hours). SCF starved cells were plated at a density of 15,000 cells per well of a 96-well plate in media without SCF. C-Kit antibodies (SR1 antibodies or

CDX-0159) or controls were titrated onto cells and the plate was incubated for 2 hours at 37 °C, 5% CO_2. Following the 2-hour incubation, SCF was added to a final concentration of 50ng/mL and plates were incubated for 72 hours at 37 °C, 5% CO_2. Cell viability was measured using Cell Titer Glo (Promega). Cell Titer Glo was added to each well and the plate was incubated for 2 minutes protected from light on a benchtop shaker. Luminescence was measured on a plate reader (PerkinElmer). Data were analyzed using GraphPad Prism.

[0251] Exemplary results are depicted in **FIG. 1**, in which antibody concentrations were plotted as a function of relative luminescence and curve fitting was performed using a four-parameter non-linear regression algorithm. IC_{50} values were determined and summarized in **Table 8**. Tested SR1 antibodies inhibited SCF-dependent proliferation in M-07e cells.

Table 8. Inhibition of SCF-dependent proliferation of M-07e cells

Antibody	IC_{50} (nM)
muSR1	0.2828
hSR1-1	0.1415
hSR1-2	0.1417
hSR1-3	0.1129
hSR1-4	0.1385
hSR1-5	0.1415
hSR1-6	0.1517
chSR1	0.2769
CDX-0159	0.4082

Example 7. Inhibition of cell proliferation by c-Kit/siglec 6 bispecific antibodies

[0252] The ability of c-Kit/siglec-6 antibodies to inhibit SCF-dependent cell proliferation was assessed.

[0253] M-07e cells were starved of SCF overnight (16 hours). SCF starved cells were plated at a density of 15,000 cells per well of a 96-well plate in media without SCF. Anti-siglec-6 JML-1 and hRND-3, anti-c-Kit chSR1 and CDX-0159, and anti-siglec/ anti-c-Kit bispecific JML-1/hSR1-1 and hRND-3/hSR1-1 were titrated onto cells and incubated for 2 hours at 37°C, 5%CO_2. Following the 2-hour incubation, SCF was added to a final concentration of 50 ng/mL and plates were incubated for 72 hours at 37 °C, 5% CO_2. Cell viability was measured using Cell Titer Glo (Promega). Cell Titer Glo was added to each well and the plate was incubated for 2 minutes protected from light on a benchtop shaker. Luminescence was measured on a plate reader (PerkinElmer). Data were analyzed using GraphPad Prism with curve fitting performed using a four-parameter non-linear regression algorithm. Relative proliferation was calculated by comparing the mean relative fluorescence

units (RFU) from each sample (cells + antibody + SCF) to mean RFU of cells incubated with SCF alone.

[0254] Results are shown in **FIG. 2**. Tested c-Kit/siglec-6 bispecific antibodies inhibited SCF-dependent proliferation in M-07e cells; siglect-6 monospecific antibodies did not inhibit proliferation.

Example 8. Mast cell degranulation assay

[0255] Experiment was performed to evaluate whether disclosed siglect-6 antibodies induce mast cell degranulation.

[0256] CD34+ progenitor cells from whole blood were cultured in Mast Cell Media containing cytokines and growth factors. Cultures matured into mast cells in 6-8 weeks from the start of the differentiation process.

[0257] To induce FcγRI expression in mast cells, cells were treated with IFN-γ at 100UI/mL for 72 hours. Mast cells with and without IFN-γ treatment were starved of cytokines for 4 hours. Cells were washed in Tyrode's buffer and plated at 35,000 cells per well in a 96-well plate. The plate was incubated for 30 minutes at 37 °C and 5% CO_2. Siglec 6 antibodies with normal human IgG1 Fc, effector slient IgG1 Fc (containing L234A/L235A/P329G mutations), or effector enhanced IgG1 Fc (afucosylated) and PMA/Ionomycin were added to appropriate wells along with SCF and the plate incubated for 30 minutes at 37°C (in air). Media from the treated mast cell cultures was added to a 96 well plate containing PNAG solution (0.35% p-nitrophenyl N-acetyl-b-D-glucosamide) and incubated for 90 minutes at 37°C (in air). Similarly, the remaining mast cells were lysed in 0.1% triton X-100 and added to PNAG solution for 90 minutes. Glycine buffer (400mM, pH10.7) was added to all wells and absorbance at 405nm was read in a plate reader. β-Hexosaminidase was measured by calculating the percent β-hexosaminidase released from the β-hexosaminidase contained by mast cells.

[0258] Results are shown in **FIG. 3**. All three Siglec-6 hRND-3 antibodies with modifications in their Fc domains did not induce degranulation of CD34 derived Mast cells

SEQUENCE ANNEX

[0259] In some sequences, variable regions are bolded, and CDRs are underlined.

Table 9. Sequences of exemplary antigen-binding sites capable of binding siglect-6

SEQ ID NO	Description	Sequence
Murine anti-siglec-6 RND (mRND)		
1	Heavy chain	QVQLQQPGAELVKPGASVKLSCKASGYAFTSYWMHWVKLRPGQGFEWI GEIYPTNGGTTYNEKFKRKATLTVDKSSSTAYMQLSSLTSEDSAVYYC TIEDFYAMDYWGQGTSVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGC LVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTW PSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSV FIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQ TQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTI SKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNN GKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGL HNHHTTKSFSRTPGK
2	Heavy chain variable region	QVQLQQPGAELVKPGASVKLSCKASGYAFTSYWMHWVKLRPGQGFEWI GEIYPTNGGTTYNEKFKRKATLTVDKSSSTAYMQLSSLTSEDSAVYYC TIEDFYAMDYWGQGTSVTVSS
3	HCDR1 (Kabat)	SYWMH
4	HCDR2 (Kabat)	EIYPTNGGTTYNEKFKR
5	HCDR3 (Kabat)	EDFYAMDY
6	HCDR1 (AbM)	GYAFTSYWMH
7	HCDR2 (AbM)	EIYPTNGGTT
8	HCDR3 (AbM)	EDFYAMDY
9	Light chain	DVVMTQTPLTLSVTIGQPASISCKSSQSLLDSDGKTCLNWLLQRPGQS PKRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVYYCWQG THFPYTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNF YPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYE RHNSYTCEATHKTSTSPIVKSFNRNEC
10	Light chain variable region	DVVMTQTPLTLSVTIGQPASISCKSSQSLLDSDGKTCLNWLLQRPGQS PKRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVYYCWQG THFPYTFGGGTKLEIKR
11	LCDR1 (Kabat)	KSSQSLLDSDGKTCLN
12	LCDR2 (Kabat)	LVSKLDS
13	LCDR3 (Kabat)	WQGTHFPYT
14	LCDR1 (AbM)	KSSQSLLDSDGKTCLN
15	LCDR2 (AbM)	LVSKLDS
16	LCDR3 (AbM)	WQGTHFPYT
Chimeric anti-siglec-6 RND (chRND)		
17	Heavy chain (*Fc or constant region may be of any IgG isotype*)	**QVQLQQPGAELVKPGASVKLSCKASGYAFTSYWMHWVKLRPGQGFEWI GEIYPTNGGTTYNEKFKRKATLTVDKSSSTAYMQLSSLTSEDSAVYYC TIEDFYAMDYWGQGTSVTVSS**ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK
18	Light chain	**DVVMTQTPLTLSVTIGQPASISCKSSQSLLDSDGKTCLNWLLQRPGQS PKRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVYYCWQG THFPYTFGGGTKLEIKR**TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
Humanized anti-siglec-6 RND heavy chain 1 (hRND-H1)		

19	hRND-H1, IgG1 (*Fc or constant region may be of any IgG isotype*)	**QVQLVQSGAEVKKPGASVKVSCKASGYAFTSYWMHWVRQAPGQRLEWM GEIYPTNGGTTYAQKFQGRVTMTRDTSTSTVYMELSRLRSDDTAVYYC AREDFYAMDYWGQGTTVTVSS**ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG
20	Heavy chain variable region	QVQLVQSGAEVKKPGASVKVSCKASGYAFTSYWMHWVRQAPGQRLEWM GEIYPTNGGTTYAQKFQGRVTMTRDTSTSTVYMELSRLRSDDTAVYYC AREDFYAMDYWGQGTTVTVSS
3	HCDR1 (Kabat)	SYWMH
21	HCDR2 (Kabat)	EIYPTNGGTTY*AQ*KF*QG*
5	HCDR3 (Kabat)	EDFYAMDY
6	HCDR1 (AbM)	GYAFTSYWMH
7	HCDR2 (AbM)	EIYPTNGGTT
8	HCDR3 (AbM)	EDFYAMDY
Humanized anti-siglec-6 RND heavy chain 2 (hRND-H2)		
22	hRND-H2, IgG1 (*Fc or constant region may be of any IgG isotype*)	**QVQLVQSGAEVKKPGASVKVSCKASGYAFTSYWMHWVRQAPGQRLEWM GEIYPTNGGTTYAQKFQGRVTMTVDKSTSTAYMELSRLRSDDTAVYYC TIEDFYAMDYWGQGTTVTVSS**ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG
23	Heavy chain variable region	QVQLVQSGAEVKKPGASVKVSCKASGYAFTSYWMHWVRQAPGQRLEWM GEIYPTNGGTTYAQKFQGRVTMTVDKSTSTAYMELSRLRSDDTAVYYC TIEDFYAMDYWGQGTTVTVSS
3	HCDR1 (Kabat)	SYWMH
21	HCDR2 (Kabat)	EIYPTNGGTTY*AQ*KF*QG*
5	HCDR3 (Kabat)	EDFYAMDY
6	HCDR1 (AbM)	GYAFTSYWMH
7	HCDR2 (AbM)	EIYPTNGGTT
8	HCDR3 (AbM)	EDFYAMDY
Humanized anti-siglec-6 RND heavy chain 3 (hRND-H3)		
24	huRND-H3, IgG1 (*Fc or constant region may be of any IgG isotype*)	QVQLVQSGAEVKKPGASVKLSCKASGYAFTSYWMHWVRQAPGQRLEWI GEIYPTNGGTTYAQKFQGRATLTVDKSTSTAYMELSRLRSDDTAVYYC TIEDFYAMDYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG
25	Heavy chain variable region	QVQLVQSGAEVKKPGASVKLSCKASGYAFTSYWMHWVRQAPGQRLEWI GEIYPTNGGTTYAQKFQGRATLTVDKSTSTAYMELSRLRSDDTAVYYC TIEDFYAMDYWGQGTTVTVSS
3	HCDR1 (Kabat)	SYWMH

21	HCDR2 (Kabat)	EIYPTNGGTTY*AQKFQG*
5	HCDR3 (Kabat)	EDFYAMDY
6	HCDR1 (AbM)	GYAFTSYWMH
7	HCDR2 (AbM)	EIYPTNGGTT
8	HCDR3 (AbM)	EDFYAMDY
Humanized anti-siglec-6 RND heavy chain 2b (hRND-H2b)		
26	hRND-H2b, IgG1 (*Fc or constant region may be of any IgG isotype*)	QVQLVQSGAEVKKPGASVKVSCKASGYAFTSYWMHWVRQAPGQRLEWM GEIYPTNGGTTYNEKFKRRVTMTVDKSTSTAYMELSRLRSDDTAVYYC TIEDFYAMDYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG
27	Heavy chain variable region	QVQLVQSGAEVKKPGASVKVSCKASGYAFTSYWMHWVRQAPGQRLEWM GEIYPTNGGTTYNEKFKRRVTMTVDKSTSTAYMELSRLRSDDTAVYYC TIEDFYAMDYWGQGTTVTVSS
3	HCDR1 (Kabat)	SYWMH
4	HCDR2 (Kabat)	EIYPTNGGTTYNEKFKR
5	HCDR3 (Kabat)	EDFYAMDY
6	HCDR1 (AbM)	GYAFTSYWMH
7	HCDR2 (AbM)	EIYPTNGGTT
8	HCDR3 (AbM)	EDFYAMDY
Humanized anti-siglec-6 RND heavy chain 3b (hRND-H3b)		
28	hRND-H3b, IgG1 (*Fc or constant region may be of any IgG isotype*)	QVQLVQSGAEVKKPGASVKLSCKASGYAFTSYWMHWVRQAPGQRLEWI GEIYPTNGGTTYNEKFKRRATLTVDKSTSTAYMELSRLRSDDTAVYYC TIEDFYAMDYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG
29	Heavy chain variable region	QVQLVQSGAEVKKPGASVKLSCKASGYAFTSYWMHWVRQAPGQRLEWI GEIYPTNGGTTYNEKFKRRATLTVDKSTSTAYMELSRLRSDDTAVYYC TIEDFYAMDYWGQGTTVTVSS
3	HCDR1 (Kabat)	SYWMH
4	HCDR2 (Kabat)	EIYPTNGGTTYNEKFKR
5	HCDR3 (Kabat)	EDFYAMDY
6	HCDR1 (AbM)	GYAFTSYWMH
7	HCDR2 (AbM)	EIYPTNGGTT
8	HCDR3 (AbM)	EDFYAMDY
Humanized anti-siglec-6 RND light chain 1 (hRND-L1)		
30	hRND-L1	DIVMTQTPLSLSVTPGQPASISCKSSQSLLDSDGKTSLNWYLQKPGQS PQLLIYLVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCWQG THFPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
31	Light chain variable region	DIVMTQTPLSLSVTPGQPASISCKSSQSLLDSDGKTSLNWYLQKPGQS PQLLIYLVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCWQG THFPYTFGQGTKLEIK

32	LCDR1 (Kabat)	KSSQSLLDSDGKT*S*LN
12	LCDR2 (Kabat)	LVSKLDS
13	LCDR3 (Kabat)	WQGTHFPYT
34	LCDR1 (AbM)	KSSQSLLDSDGKT*S*LN
15	LCDR2 (AbM)	LVSKLDS
16	LCDR3 (AbM)	WQGTHFPYT
Humanized anti-siglec-6 RND light chain 2 (hRND-L2)		
35	hRND-L2	DIVMTQTPLSLSVTPGQPASISCKSSQSLLDSDGKTSLNWLLQKPGQS PQLLIYLVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCWQG THFPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
36	Light chain variable region	DIVMTQTPLSLSVTPGQPASISCKSSQSLLDSDGKTSLNWLLQKPGQS PQLLIYLVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCWQG THFPYTFGQGTKLEIK
32	LCDR1 (Kabat)	KSSQSLLDSDGKT*S*LN
12	LCDR2 (Kabat)	LVSKLDS
13	LCDR3 (Kabat)	WQGTHFPYT
34	LCDR1 (AbM)	KSSQSLLDSDGKT*S*LN
15	LCDR2 (AbM)	LVSKLDS
16	LCDR3 (AbM)	WQGTHFPYT
Humanized anti-siglec-6 RND light chain 3 (hRND-L3)		
37	hRND-L3	DIVMTQTPLSLSVTPGQPASISCKSSQSLLDSDGKTSLNWLLQKPGQS PQRLIYLVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCWQG THFPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
38	Light chain variable region	DIVMTQTPLSLSVTPGQPASISCKSSQSLLDSDGKTSLNWLLQKPGQS PQRLIYLVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCWQG THFPYTFGQGTKLEIK
32	LCDR1 (Kabat)	KSSQSLLDSDGKT*S*LN
12	LCDR2 (Kabat)	LVSKLDS
13	LCDR3 (Kabat)	WQGTHFPYT
34	LCDR1 (AbM)	KSSQSLLDSDGKT*S*LN
15	LCDR2 (AbM)	LVSKLDS
16	LCDR3 (AbM)	WQGTHFPYT
Human anti-siglec JML-1		
120	Heavy chain, IgG1 (*Fc or constant region may be of any IgG isotype*)	KVQLLESGGGLVQPGRSLRLSCAASGFTFDDYGMHWVRQAPGKGLEWV SGISWNSGSIGYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC ARGGQTIDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG
121	Heavy chain variable region	KVQLLESGGGLVQPGRSLRLSCAASGFTFDDYGMHWVRQAPGKGLEWV SGISWNSGSIGYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC ARGGQTIDIWGQGTMVTVSS
122	HCDR1 (Kabat)	DYGMH
123	HCDR2 (Kabat)	GISWNSGSIGYADSVKG
124	HCDR3 (Kabat)	GGQTIDI

125	Light chain	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPF TFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
126	Light chain variable region	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPF TFGPGTKVDIK
127	LCDR1 (Kabat)	RASQSISSYLN
128	LCDR2 (Kabat)	AASSLQS
129	LCDR3 (Kabat)	QQSYSTPFT
Murine anti-siglec-6 M2 (mM2)		
132	Heavy chain variable region	EVQLQQSVAELVRPGASVKLSCTGSGFNIENTYTHWVKQRPEQGLEWI GRIDPANTNTKYAPKFQGKATITADTSSNTAFLQLSSLTSEDTAIYYC ALYDEDFWGPGTTLTVSS
133	HCDR1 (Kabat)	NTYTH
134	HCDR2 (Kabat)	RIDPANTNTKYAPKFQG
135	HCDR3 (Kabat)	YDEDF
136	Light chain variable region	QIVLTQSPAIMSASPGEKVTMTCSASSSLTYMHWYQQKSGTSPKRWIY DTSELASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWNSDPPT FGGGTKLEIK
137	LCDR1 (Kabat)	SASSSLTYMH
138	LCDR2 (Kabat)	DTSELAS
139	LCDR3 (Kabat)	QQWNSDPPT
Murine anti-siglec-6 M7 (mM7)		
140	Heavy chain variable region	EFQLQQSGPELVKPGASVKISCKASDYSFTDYNMNWVKQSNGKSLEWI GVINPNYGTTSYNQKFKGKATLTVDQSSSTAYMQLNSLTSEDSAVYYC ARSGGFSWGQGTLVTVSA
141	HCDR1 (Kabat)	DYNMN
142	HCDR2 (Kabat)	VINPNYGTTSYNQKFKG
143	HCDR3 (Kabat)	SGGFS
144	Light chain variable region	DIVMTQSHTFMSTSVGDRVSITCKASQDVSTAVAWYQQKPGQSPKLLI FSASYRYTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYYCQQHYSTPT WTFGGGTKLEIK
145	LCDR1 (Kabat)	KASQDVSTAVA
146	LCDR2 (Kabat)	SASYRYT
147	LCDR3 (Kabat)	QQHYSTPTWT
Murine anti-siglec-6 M11 (mM11)		
148	Heavy chain variable region	EVQLVESGGGLVQPKGSLKLSCAASGFTFKNYVMHWVRQTPGKGLEWV ARIRSKNSNYATYYADSVKDRFTISRDDSQSMLYLQMNNLKTEDTAMY YCMRALVAYWGQGTLVTVSA
149	HCDR1 (Kabat)	NYVMH
150	HCDR2 (Kabat)	RIRSKNSNYATYYADSVKD
151	HCDR3 (Kabat)	ALVAY
152	Light chain variable region	QLVLTQSPAIMSTSPGEKVTMTCSASSSVSYIYWYQQKPGSSPRLLIY DTSNLASGVPVRFSGSGSGTSYSLTISRMEAEDAATYYCQQWSSYPYT FGGGTKLEIK
153	LCDR1 (Kabat)	SASSSVSYIY
154	LCDR2 (Kabat)	DTSNLAS
155	LCDR3 (Kabat)	QQWSSYPYT
Chimeric anti-siglec-6 M11 (chM11)		
156	Heavy chain	EVQLVESGGGLVQPKGSLKLSCAASGFTFKNYVMHWVRQTPGKGLEWV ARIRSKNSNYATYYADSVKDRFTISRDDSQSMLYLQMNNLKTEDTAMY

	(*Fc or constant region may be of any IgG isotype*)	YCMRALVAYWGQGTLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
157	Light chain	QLVLTQSPAIMSTSPGEKVTMTCSASSSVSYIYWYQQKPGSSPRLLIY DTSNLASGVPVRFSGSGSGTSYSLTISRMEAEDAATYYCQQWSSYPYT FGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
Humanized anti-siglec-6 M11 heavy chain 1 (hM11-H1)		
158	hM11-H1, IgG1 (*Fc or constant region may be of any IgG isotype*)	**EVQLVESGGGLVQPGGSLRLSCAASGFTFKNYVMHWVRQAPGKGLEWV SRIRSKNSNYATYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVY YCAKALVAYWGQGTLVTVSS**ASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
159	Heavy chain variable region	EVQLVESGGGLVQPGGSLRLSCAASGFTFKNYVMHWVRQAPGKGLEWV SRIRSKNSNYATYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVY YCAKALVAYWGQGTLVTVSS
149	HCDR1 (Kabat)	NYVMH
160	HCDR2 (Kabat)	RIRSKNSNYATYYADSVK*G*
151	HCDR3 (Kabat)	ALVAY
Humanized anti-siglec-6 M11 heavy chain 2 (hM11-H2)		
161	hM11-H2, IgG1 (*Fc or constant region may be of any IgG isotype*)	**EVQLVESGGGLVQPGGSLRLSCAASGFTFKNYVMHWVRQAPGKGLEWV ARIRSKNSNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVY YCMRALVAYWGQGTLVTVSS**ASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
162	Heavy chain variable region	EVQLVESGGGLVQPGGSLRLSCAASGFTFKNYVMHWVRQAPGKGLEWV ARIRSKNSNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVY YCMRALVAYWGQGTLVTVSS
149	HCDR1 (Kabat)	NYVMH
160	HCDR2 (Kabat)	RIRSKNSNYATYYADSVK*G*
151	HCDR3 (Kabat)	ALVAY
Humanized anti-siglec-6 M11 light chain 1 (hM11-L1)		
163	hM11-L1	**DIQMTQSPSSLSASVGDRVTITCRASSSVSYIYWYQQKPGKAPKLLIY DTSNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSYPYT FGQGTKLEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC

164	Light chain variable region	DIQMTQSPSSLSASVGDRVTITCRASSSVSYIYWYQQKPGKAPKLLIY DTSNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSYPYT FGQGTKLEIK
165	LCDR1 (Kabat)	*R*ASSSVSYIY
154	LCDR2 (Kabat)	DTSNLAS
155	LCDR3 (Kabat)	QQWSSYPYT
Humanized anti-siglec-6 M11 light chain 2 (hM11-L2)		
166	hM11-L2	**DIQMTQSPSSLSASVGDRVTITCRASSSVSYIYWYQQKPGKAPKLLIY DTSNLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQWSSYPYT FGQGTKLEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
167	Light chain variable region	DIQMTQSPSSLSASVGDRVTITCRASSSVSYIYWYQQKPGKAPKLLIY DTSNLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQWSSYPYT FGQGTKLEIK
165	LCDR1 (Kabat)	*R*ASSSVSYIY
154	LCDR2 (Kabat)	DTSNLAS
155	LCDR3 (Kabat)	QQWSSYPYT
Humanized anti-siglec-6 M11 light chain 3 (hM11-L3)		
168	hM11-L3	**DIQLTQSPSSLSASVGDRVTMTCRASSSVSYIYWYQQKPGKAPKLLIY DTSNLASGVPSRFSGSGSGTDYTLTISSMQPEDFATYYCQQWSSYPYT FGQGTKLEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
169	Light chain variable region	DIQLTQSPSSLSASVGDRVTMTCRASSSVSYIYWYQQKPGKAPKLLIY DTSNLASGVPSRFSGSGSGTDYTLTISSMQPEDFATYYCQQWSSYPYT FGQGTKLEIK
165	LCDR1 (Kabat)	*R*ASSSVSYIY
154	LCDR2 (Kabat)	DTSNLAS
155	LCDR3 (Kabat)	QQWSSYPYT

Table 10. Sequences of exemplary antigen-binding sites capable of binding c-Kit

SEQ ID NO	**Description**	**Sequence**
Murine anti-c-Kit SR1 (mSR1)		
39	Heavy chain	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWI GVIYSGNGDTSYNQKFKGKATLTADKSSSTAYMQINSLTSEDSAVYYC ARERDTRFGNWGQGTLVTVSAAKTTAPSVYPLAPVCGDTTGSSVTLGC LVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTW PSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSV FIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQ TQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTI SKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNN GKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGL HNHHTTKSFSRTPGK
40	Heavy chain variable region	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWI GVIYSGNGDTSYNQKFKGKATLTADKSSSTAYMQINSLTSEDSAVYYC ARERDTRFGNWGQGTLVTVSA
41	HCDR1 (Kabat)	SYNMH
42	HCDR2 (Kabat)	VIYSGNGDTSYNQKFKG
43	HCDR3 (Kabat)	ERDTRFGN
44	Light chain	NIVLTQSPASLAVSLGLRATISCRASESVDIYGNSFMHWYQQKPGQPP KLLIYLASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNN

		EDPYTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFY PKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYER HNSYTCEATHKTSTSPIVKSFNRNEC
45	Light chain variable region	NIVLTQSPASLAVSLGLRATISCRASESVDIYGNSFMHWYQQKPGQPP KLLIYLASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNN EDPYTFGGGTKLEIK
46	LCDR1 (Kabat)	RASESVDIYGNSFMH
47	LCDR2 (Kabat)	LASNLES
48	LCDR3 (Kabat)	QQNNEDPYT
Chimeric anti-c-Kit SR1 (chSR1)		
49	Heavy chain (*Fc or constant region may be of any IgG isotype*)	**QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWI GVIYSGNGDTSYNQKFKGKATLTADKSSSTAYMQINSLTSEDSAVYYC ARERDTRFGNWGQGTLVTVSA**ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK
50	Light chain	**NIVLTQSPASLAVSLGLRATISCRASESVDIYGNSFMHWYQQKPGQPP KLLIYLASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNN EDPYTFGGGTKLEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
Humanized anti-c-Kit SR1 heavy chain 2 (hSR1-H2)		
51	hSR1-H2, IgG1 (*Fc or constant region may be of any IgG isotype*)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSGNGDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK
52	Heavy chain variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSGNGDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
41	HCDR1 (Kabat)	SYNMH
53	HCDR2 (Kabat)	VIYSGNGDTSYAQKFQG
43	HCDR3 (Kabat)	ERDTRFGN
Humanized anti-c-Kit SR1 heavy chain 3 (hSR1-H3)		
54	hSR1-H3, IgG1 (*Fc or constant region may be of any IgG isotype*)	QVQLVQSGAEVKKPGASVKMSCKASGYTFTSYNMHWVRQAPGQGLEWI GVIYSGNGDTSYAQKFQGRATLTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK

55	Heavy chain variable region	QVQLVQSGAEVKKPGASVKMSCKASGYTFTSYNMHWVRQAPGQGLEWI GVIYSGNGDTSYAQKFQGRATLTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
41	HCDR1 (Kabat)	SYNMH
53	HCDR2 (Kabat)	VIYSGNGDTSYAQKFQG
43	HCDR3 (Kabat)	ERDTRFGN
Humanized anti-c-Kit SR1 heavy chain 2b (hSR1-H2b)		
56	hSR1-H2b, IgG1 (*Fc or constant region may be of any IgG isotype*)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSGNGDTSYAQKFQGRVTMTADKSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK
57	Heavy chain variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSGNGDTSYAQKFQGRVTMTADKSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
41	HCDR1 (Kabat)	SYNMH
53	HCDR2 (Kabat)	VIYSGNGDTSYAQKFQG
43	HCDR3 (Kabat)	ERDTRFGN
Humanized anti-c-Kit SR1 heavy chain 3b (hSR1-H3b)		
58	hSR1-H3b, IgG1 (*Fc or constant region may be of any IgG isotype*)	**QVQLVQSGAEVKKPGASVKMSCKASGYTFTSYNMHWVRQAPGQGLEWI GVIYSGNGDTSYAQKFQGRATLTADKSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS**ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK
59	Heavy chain variable region	**QVQLVQSGAEVKKPGASVKMSCKASGYTFTSYNMHWVRQAPGQGLEWI GVIYSGNGDTSYAQKFQGRATLTADKSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS**
41	HCDR1 (Kabat)	SYNMH
53	HCDR2 (Kabat)	VIYSGNGDTSYAQKFQG
43	HCDR3 (Kabat)	ERDTRFGN
hSR1-H2 anti-c-Kit variants		
60	HCDR1 (Kabat) variant 1	SY*T*MH
61	HCDR1 (Kabat) variant 2	SY*Q*MH
62	HCDR1 (Kabat) variant 3	*N*YNMH
63	HCDR2 (Kabat) variant 1	VIYSG*S*GDTSYAQKFQG
64	HCDR2 (Kabat) variant 2	VIYSG*Q*GDTSYAQKFQG
65	HCDR2 (Kabat) variant 3	VIYSGNG*S*TSYAQKFQG
66	HCDR2 (Kabat) variant 4	VIYSGNG*E*TSYAQKFQG
67	HCDR2 (Kabat) variant 5	VI*F*SGNGDTSYAQKFQG
68	HCDR2 (Kabat) variant 6	VI*H*SGNGDTSYAQKFQG
69	HCDR3 (Kabat) variant 1	ER*E*TRFGN
70	HCDR3 (Kabat) variant 2	ER*S*TRFGN
71	HCDR3 (Kabat) variant 3	E*K*DTRFGN
72	HCDR3 (Kabat) variant 4	ERDT*K*FGN

73	hSR1-H2 N33T variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSY**T**MHWVRQAPGQGLEWM GVIYSGNGDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
74	hSR1-H2 N33Q variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSY**Q**MHWVRQAPGQGLEWM GVIYSGNGDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
75	hSR1-H2 N31N variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFT**N**YNMHWVRQAPGQGLEWM GVIYSGNGDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
76	hSR1-H2 N54S variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSG**S**GDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
77	hSR1-H2 N54Q variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSG**Q**GDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
78	hSR1-H2 N56S variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSGNG**S**TSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
79	hSR1-H2 N56E variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSGNG**E**TSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
80	hSR1-H2 N52F variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVI**F**SGNGDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
81	hSR1-H2 N52H variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVI**H**SGNGDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDTRFGNWGQGTLVTVSS
82	hSR1-H2 N97E variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSGNGDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARER**E**TRFGNWGQGTLVTVSS
83	hSR1-H2 N97S variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSGNGDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARER**S**TRFGNWGQGTLVTVSS
84	hSR1-H2 N96K variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSGNGDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARE**K**DTRFGNWGQGTLVTVSS
85	hSR1-H2 N99K variable region	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWM GVIYSGNGDTSYAQKFQGRVTMTADTSTSTAYMELSSLRSEDTAVYYC ARERDT**K**FGNWGQGTLVTVSS
Humanized anti-c-Kit SR1 light chain 2 (hSR1-L2)		
86	hSR1-L2	DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN EDPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
87	Light chain variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN EDPYTFGQGTKLEIK
46	LCDR1 (Kabat)	RASESVDIYGNSFMH
47	LCDR2 (Kabat)	LASNLES
48	LCDR3 (Kabat)	QQNNEDPYT
Humanized anti-c-Kit SR1 light chain 2r (hSR1-L2r)		
88	hSR1-L2r	**DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSRTDFTLTISSVQPEDFATYYCQQNN EDPYTFGQGTKLEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY

		PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
89	Light chain variable region	**DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSRTDFTLTISSVQPEDFATYYCQQNN EDPYTFGQGTKLEIK**
46	LCDR1 (Kabat)	RASESVDIYGNSFMH
47	LCDR2 (Kabat)	LASNLES
48	LCDR3 (Kabat)	QQNNEDPYT
hSR1-L2 variants		
90	LCDR1 (Kabat) variant 1	RASESVD*S*YGNSFMH
91	LCDR1 (Kabat) variant 2	RASESVD*N*YGNSFMH
92	LCDR1 (Kabat) variant 3	RASESVDI*F*GNSFMH
93	LCDR1 (Kabat) variant 4	RASESVDI*H*GNSFMH
94	LCDR1 (Kabat) variant 5	RASESVDIYG*S*SFMH
95	LCDR1 (Kabat) variant 6	RASESVDIYG*Q*SFMH
96	LCDR2 (Kabat) variant 1	*A*ASNLES
97	LCDR2 (Kabat) variant 2	LAS*S*LES
98	LCDR3 (Kabat) variant 1	QQN*Q*EDPYT
99	LCDR3 (Kabat) variant 2	QQN*S*EDPYT
100	LCDR3 (Kabat) variant 3	QQNN*D*DPYT
101	LCDR3 (Kabat) variant 4	QQNN*S*DPYT
102	LCDR3 (Kabat) variant 5	QQNNE*E*PYT
103	LCDR3 (Kabat) variant 6	QQNNE*S*PYT
104	LCDR3 (Kabat) variant 7	QQNNEDP*F*T
105	hSR1-L2 I30aS variable region	DIQLTQSPSSLSASVGDRATITCRASESVD*S*YGNSFMHWYQQKP GKAPKLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFA TYYCQQNNEDPYTFGQGTKLEIK
106	hSR1-L2 I30aN variable region	DIQLTQSPSSLSASVGDRATITCRASESVD*N*YGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN EDPYTFGQGTKLEIK
107	hSR1-L2 Y30bF variable region	DIQLTQSPSSLSASVGDRATITCRASESVDI*F*GNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN EDPYTFGQGTKLEIK
108	hSR1-L2 Y30bH variable region	DIQLTQSPSSLSASVGDRATITCRASESVDI*H*GNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN EDPYTFGQGTKLEIK
109	hSR1-L2 N30dS variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYG*S*SFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN EDPYTFGQGTKLEIK
110	hSR1-L2 N30dQ variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYG*Q*SFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN EDPYTFGQGTKLEIK
111	hSR1-L2 L50A variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKP GKAPKLLIY*A*ASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFA TYYCQQNNEDPYTFGQGTKLEIK
112	hSR1-L2 N53S variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLAS*S*LESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN EDPYTFGQGTKLEIK
113	hSR1-L2 N92Q variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQN*Q* EDPYTFGQGTKLEIK
114	hSR1-L2 N92S variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQN*S* EDPYTFGQGTKLEIK

115	hSR1-L2 E93D variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN ⍰DPYTFGQGTKLEIK
116	hSR1-L2 E93S variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN ⍰DPYTFGQGTKLEIK
117	hSR1-L2 D94E variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN E⍰PYTFGQGTKLEIK
118	hSR1-L2 D94S variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN E⍰PYTFGQGTKLEIK
119	hSR1-L2 Y96F variable region	DIQLTQSPSSLSASVGDRATITCRASESVDIYGNSFMHWYQQKPGKAP KLLIYLASNLESGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQNN EDP⍰TFGQGTKLEIK

Table 11. Other sequences

SEQ ID NO	Description	Sequence
130	Human immunoglobulin G1 (IgG1) heavy chain constant (CH) region	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
131	Human immunoglobulin kappa constant region	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC

INCORPORATION BY REFERENCE

[0260] Unless stated to the contrary, the entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS/ OTHER EMBODIMENTS

[0261] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments described herein. Such equivalents are intended to be encompassed by the following claims.

WHAT IS CLAIMED IS:

1. An antibody or antigen-binding fragment thereof, comprising a first antigen-binding domain that is capable of binding siglec-6 and a second antigen-binding domain that is capable of binding c-Kit.

2. The antibody or antigen-binding fragment thereof of claim **1**, wherein the first antigen-binding domain is capable of binding siglec-6 with a higher affinity than the second antigen-binding domain is capable of binding c-Kit.

3. The antibody or antigen-binding fragment thereof of claim **1**, wherein the first antigen-binding domain is capable of binding siglec-6 with an affinity characterized by a K_D value that is lower than about 100 uM, lower than about 100 nM, lower than about 10 nM, lower than about 1 nM, lower than about 100 pM, lower than about 10 pM, lower than about 1 pM, lower than about 100 fM, or lower than about 10 fM.

4. The antibody or antigen-binding fragment thereof of claim **3**, wherein the first antigen-binding domain is capable of binding c-Kit with an affinity characterized by a K_D value that is between about 1 nM and about 900 uM, between about 5 nM and about 500 uM, between about 10 nM and about 100uM, between about 20 nM and about 10 10 uM, or between about 25 and about 500 nM.

5. The antibody or antigen-binding fragment thereof of any one of claims **1-4**, wherein the antibody or antigen-binding fragment is capable of binding a cell expressing siglect-6.

6. The antibody or antigen-binding fragment thereof of any one of claims **1-5**, wherein the antibody or antigen-binding fragment is capable of binding a mast cell.

7. The antibody or antigen-binding fragment thereof of claim **6**, wherein the antibody or antigen-binding fragment binds a mast cell preferentially than another immune cell.

8. The antibody or antigen-binding fragment thereof of claim **6**, wherein the immune cell is T cell, dendritic cell, macrophage, neutrophil, or basophil.

9. The antibody or antigen-binding fragment thereof of any one of claims **1-8**, wherein the antibody or antigen-binding fragment thereof does not induce mast cell degranulation.

10. The antibody or antigen-binding fragment thereof of any one of claims **1-9**, wherein the antibody or antigen-binding fragment thereof has a comparable or lower internalization rate compared to a reference antibody capable of binding siglect-6.

11. The antibody or antigen-binding fragment thereof of claim **10**, wherein the reference antibody capable of binding siglect-6 comprises a heavy chain variable (VH) region comprising the amino acid sequence of SEQ ID NO: 2, and a light chain variable (VL) region comprising the amino acid sequence of SEQ ID NO: 10.

12. The antibody or antigen-binding fragment thereof of any one of claims **1-11**, wherein the antibody or antigen-binding fragment thereof has a comparable or lower toxicity compared to a reference antibody capable of binding c-Kit.

13. The antibody or antigen-binding fragment thereof of claim **12**, wherein the reference antibody capable of binding c-Kit comprises a VH region comprising the amino acid sequence of SEQ ID NO: 40, and a VL region comprising the amino acid sequence of SEQ ID NO: 45.

14. The antibody or antigen-binding fragment thereof of any one of claims **1-13**, wherein the first antigen-binding domain comprises:

(a) a VH region comprising:

heavy chain complementarity-determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 3, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 4 or 21, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 5, or a sequence differing in 1 or 2 amino acids therefrom; and

(b) a VL region comprising:

heavy chain complementarity-determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 11 or 32, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 12, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 13, or a sequence differing in 1 or 2 amino acids therefrom.

15. The antibody or antigen-binding fragment thereof of claim **14**, wherein the first antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 3,

HCDR2 having the amino acid sequence of SEQ ID NO: 4 or 21, and

HCDR3 having the amino acid sequence of SEQ ID NO: 5; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 11 or 32,

LCDR2 having the amino acid sequence of SEQ ID NO: 12, and

LCDR3 having the amino acid sequence of SEQ ID NO: 13.

16. The antibody or antigen-binding fragment thereof of claim **14**, wherein the first antigen-binding domain comprises:

(a) a VH region comprising:

(i) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 3, 4, and 5, respectively; or

(ii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 3, 21, and 5, respectively; and

(b) a VL region comprising:

(i) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 11, 12, and 13, respectively; or

(ii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 32, 12, and 13, respectively.

17. The antibody or antigen-binding fragment thereof of any one of claims **14-16**, wherein the first antigen-binding domain comprises:

(a) a VH region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 2, 20, 23, 25, 27, or 29; and

(b) a VL region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10, 31, 36, or 38.

18. The antibody or antigen-binding fragment thereof of claim **17**, wherein the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 2, 20, 23, 25, 27, or 29; and

(b) a VL region having the amino acid sequence of SEQ ID NO: 10, 31, 36, or 38.

19. The antibody or antigen-binding fragment thereof of claim **17** or **18**, wherein the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 2, and a VL region having the amino acid sequence of SEQ ID NO: 10;

(b) a VH region having the amino acid sequence of SEQ ID NO: 23, and a VL region having the amino acid sequence of SEQ ID NO: 38;

(c) a VH region having the amino acid sequence of SEQ ID NO: 25, and a VL region having the amino acid sequence of SEQ ID NO: 38;

(d) a VH region having the amino acid sequence of SEQ ID NO: 27, and a VL region having the amino acid sequence of SEQ ID NO: 38; or

(e) a VH region having the amino acid sequence of SEQ ID NO: 29, and a VL region having the amino acid sequence of SEQ ID NO: 38.

20. The antibody or antigen-binding fragment thereof of claim **19**, wherein the first antigen-binding domain comprises:

a VH region having the amino acid sequence of SEQ ID NO: 25, and a VL region having the amino acid sequence of SEQ ID NO: 38.

21. The antibody or antigen-binding fragment thereof of any one of claims **1-13**, wherein the first antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 122, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 123, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 124, or a sequence differing in 1 or 2 amino acids therefrom; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 127, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 128, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 129, or a sequence differing in 1 or 2 amino acids therefrom.

22. The antibody or antigen-binding fragment thereof of claim **21**, wherein the first antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 122,

HCDR2 having the amino acid sequence of SEQ ID NO: 123, and

HCDR3 having the amino acid sequence of SEQ ID NO: 124; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 127,

LCDR2 having the amino acid sequence of SEQ ID NO: 128, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139.

23. The antibody or antigen-binding fragment thereof of claim **21** or **22**, wherein the first antigen-binding domain comprises:

(a) a VH region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 121; and

(b) a VL region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 126.

24. The antibody or antigen-binding fragment thereof of claim **23**, wherein the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 121; and

(b) a VL region having the amino acid sequence of SEQ ID NO: 126.

25. The antibody or antigen-binding fragment thereof of any one of claims **1-13**, wherein the first antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 133, 141, or 149, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, 142, 150, or 160, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135, 143, or 151, or a sequence differing in 1 or 2 amino acids therefrom; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 137, 145, 153, or 165, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, 146, or 154, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139, 147, or 155, or a sequence differing in 1 or 2 amino acids therefrom.

26. The antibody or antigen-binding fragment thereof of claim **25**, wherein the first antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 133, 141, or 149,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, 142, 150, or 160, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135, 143, or 151; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 137, 145, 153, or 165,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, 146, or 154, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139, 147, or 155.

27. The antibody or antigen-binding fragment thereof of claim **25** or **26**, wherein the first antigen-binding domain comprises:

(a) a VH region comprising:

(i) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 133, 134, and 135, respectively;

(ii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 141, 142, and 143, respectively;

(iii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 149, 150, and 151, respectively; or

(iv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 149, 160, and 151, respectively; and

(b) a VL region comprising:

(i) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 137, 138, and 139, respectively;

(ii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 145, 146, and 147, respectively;

(iii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 153, 154, and 155, respectively; or

(iv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 165, 154, and 155, respectively.

28. The antibody or antigen-binding fragment thereof of any one of claims **25-27**, wherein the first antigen-binding domain comprises:

(a) a VH region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 132, 140, 148, 159, or 162; and

(b) a VL region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 136, 144, 152, 164, 167, or 169.

29. The antibody or antigen-binding fragment thereof of claim **28**, wherein the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 132, 140, 148, 159, or 162; and

(b) a VL region having the amino acid sequence of SEQ ID NO: 136, 144, 152, 164, 167, or 169.

30. The antibody or antigen-binding fragment thereof of claim **28** or **29**, wherein the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 132, and a VL region having the amino acid sequence of SEQ ID NO: 139;

(b) a VH region having the amino acid sequence of SEQ ID NO: 140, and a VL region having the amino acid sequence of SEQ ID NO: 144; or

(c) a VH region having the amino acid sequence of SEQ ID NO: 148, and a VL region having the amino acid sequence of SEQ ID NO: 152.

31. The antibody or antigen-binding fragment thereof of claim **28** or **29**, wherein the first antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 159, and a VL region having the amino acid sequence of SEQ ID NO: 164;

(6) a VH region having the amino acid sequence of SEQ ID NO: 162, and a VL region having the amino acid sequence of SEQ ID NO: 167; or

(c) a VH region having the amino acid sequence of SEQ ID NO: 162, and a VL region having the amino acid sequence of SEQ ID NO: 169.

32. The antibody or antigen-binding fragment thereof of any one of claims **1-31**, wherein the second antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 41, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 42 or 53, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 43, or a sequence differing in 1 or 2 amino acids therefrom; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 46, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 47, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 48, or a sequence differing in 1 or 2 amino acids therefrom.

33. The antibody or antigen-binding fragment thereof of claim **32**, wherein the second antigen-binding domain comprises:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 41,

HCDR2 having the amino acid sequence of SEQ ID NO: 42 or 53, and

HCDR3 having the amino acid sequence of SEQ ID NO: 43; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 46,

LCDR2 having the amino acid sequence of SEQ ID NO: 47, and

LCDR3 having the amino acid sequence of SEQ ID NO: 48.

34. The antibody or antigen-binding fragment thereof of claim **32**, wherein the second antigen-binding domain comprises a VH region comprising:

(a) HCDR1 having the amino acid sequence of SEQ ID NO: 60, 61, or 62,

(b) HCDR2 having the amino acid sequence of SEQ ID NO: 63, 64, 65, 66, 67, or 68, and

(c) HCDR3 having the amino acid sequence of SEQ ID NO: 69, 70, 71, or 72.

35. The antibody or antigen-binding fragment thereof of claim **32**, wherein the second antigen-binding domain comprises a VH region comprising:

(a) LCDR1 having the amino acid sequence of SEQ ID NO: 90, 91, 92, 93, 94, or 95,

(b) LCDR2 having the amino acid sequence of SEQ ID NO: 96 or 97, and

(c) LCDR3 having the amino acid sequence of SEQ ID NO: 98, 99, 100, 101, 102, 103, or 104.

36. The antibody or antigen-binding fragment thereof of any one of claims **32-35**, wherein the second antigen-binding domain comprises:

(a) a VH region comprising:

(i) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 42, and 43, respectively;

(ii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 43, respectively;

(iii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 60, 53, and 43, respectively;

(iv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 61, 53, and 43, respectively;

(v) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 62, 53, and 43, respectively;

(vi) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 63, and 43, respectively;

(vii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 64, and 43, respectively;

(viii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 65, and 43, respectively;

(ix) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 66, and 43, respectively;

(x) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 67, and 43, respectively;

(xi) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 68, and 43, respectively;

(xii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 69, respectively;

(xiii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 70, respectively;

(xiv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 71, respectively; or

(xv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 72, respectively; and

(b) a VL region comprising:

(i) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 48, respectively;

(ii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 90, 47, and 48, respectively;

(iii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 91, 47, and 48, respectively;

(iv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 92, 47, and 48, respectively;

(v) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 93, 47, and 48, respectively;

(vi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 94, 47, and 48, respectively;

(vii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 95, 47, and 48, respectively;

(viii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 96, and 48, respectively;

(ix) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 97, and 48, respectively;

(x) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 98, respectively;

(xi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 99, respectively;

(xii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 100, respectively;

(xiii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 101, respectively;

(xiv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 102, respectively;

(xv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 103, respectively; or

(xvi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 104, respectively.

37. The antibody or antigen-binding fragment thereof of any one of claims **32-36**, wherein the second antigen-binding domain comprises:

(a) a VH region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 40, 52, 55, 57, 59, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, or 85; and

(b) a VL region comprising an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 45, 87, 89, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119.

38. The antibody or antigen-binding fragment thereof of claim **37**, wherein the second antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 40, 52, 55, 57, 59, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, or 85; and

(b) a VL region having the amino acid sequence of SEQ ID NO: 45, 87, 89, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119.

39. The antibody or antigen-binding fragment thereof of claim **37** or **38**, wherein the second antigen-binding domain comprises:

(a) a VH region having the amino acid sequence of SEQ ID NO: 40, and a VL region having the amino acid sequence of SEQ ID NO: 45;

(b) a VH region having the amino acid sequence of SEQ ID NO: 52, and a VL region having the amino acid sequence of SEQ ID NO: 87;

(c) a VH region having the amino acid sequence of SEQ ID NO: 55, and a VL region having the amino acid sequence of SEQ ID NO: 87;

(d) a VH region having the amino acid sequence of SEQ ID NO: 57, and a VL region having the amino acid sequence of SEQ ID NO: 87;

(e) a VH region having the amino acid sequence of SEQ ID NO: 59, and a VL region having the amino acid sequence of SEQ ID NO: 87;

(f) a VH region having the amino acid sequence of SEQ ID NO: 52, and a VL region having the amino acid sequence of SEQ ID NO: 89; or

(g) a VH region having the amino acid sequence of SEQ ID NO: 55, and a VL region having the amino acid sequence of SEQ ID NO: 89.

40. The antibody or antigen-binding fragment thereof of claim **39**, wherein the second antigen-binding domain comprises:

a VH region having the amino acid sequence of SEQ ID NO: 52, and a VL region having the amino acid sequence of SEQ ID NO: 87.

41. The antibody or antigen-binding fragment thereof of any one of the claims **1-40**, wherein the antibody or antigen-binding fragment thereof comprises:
(a) a first heavy chain, comprising the VH region of the first antigen-binding domain, and a first heavy chain constant (CH) region or a fragment thereof;
(b) a first light chain, comprising the VL region of the first antigen-binding domain, and a first light chain constant (CL) region or a fragment thereof;
(c) a second heavy chain, comprising the VH region of the second antigen-binding domain, and a second CH region or a fragment thereof; and
(d) a second light chain, comprising the VL region of the second antigen-binding domain, and a second CL region or a fragment thereof.

42. The antibody or antigen-binding fragment thereof of claim **41**, wherein the first and second CH region each comprise an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 130.

43. The antibody or antigen-binding fragment thereof of claim **34** or **35**, wherein the first and second heavy chain form a heterodimer, optionally wherein one of the first and second heavy chains comprises a Serine residue at position 366, an Alanine residue at position 368 and a Valine residue at position 407; and the other heavy chain comprises a Tryptophan residue at position 366, wherein the numbering of the constant region is as per the EU index.

44. The antibody or antigen-binding fragment thereof of any one of claims **41-43**, wherein the first and/or second CH region comprise one or more mutations that increase serum half-life of the antibody or antigen-binding fragment.

45. The antibody or antigen-binding fragment thereof of claim **44**, wherein the the first and/or second CH region comprises is a human IgG1 CH comprising the following mutations: M252Y, S254T, and T256E, wherein the numbering of the constant region is as per the EU index.

46. The antibody or antigen-binding fragment thereof of any one of claims **43-45**, wherein the first and/or second CH region comprise one or more mutations that reduce or abrogate effector function.

47. The antibody or antigen-binding fragment thereof of claim **46**, wherein the first and/or second CH region is a human IgG1 CH comprising one of the following mutation(s), wherein the numbering of the constant region is as per the EU index:

(a) L234A and/or L235A;

(b) A327G, A330S, and/or P331S;

(c) E233P, L234V, L235A, and/or G236del;

(d) E233P, L234V, and/or L235A;

(e) E233P, L234V, L235A, G236del, A327G, A330S, and/or P331S;

(f) E233P, L234V, L235A, A327G, A330S, and/or P331S;

(g) N297A;

(h) N297G;

(i) N297Q;

(j) L242C, N297C, and/or K334C;

(k) A287C, N297G, and/or L306C;

(l) R292C, N297G, and/or V302C;

(m) N297G, V323C, and/or I332C;

(n) V259C, N297G, and/or L306C;

(o) L234F, L235Q, K322Q, M252Y, S254T, and/or T256E;

(p) L234A, L235A, and/or P329G; or

(q) L234A, L235Q, and K322Q.

48. The antibody or antigen-binding fragment thereof of claim **46**, wherein the first and/or second CH region is a human IgG2 CH comprising one of the following mutation(s), wherein the numbering of the constant region is as per the EU index:

(a) A330S and/or P331S;

(b) V234A, G237A, P238S, H268A, V309L, A330S, and/or P331S; or

(c) V234A, G237A, H268Q, V309L, A330S, P331S, C232S, C233S, S267E, L328F, M252Y, S254T, and/or T256E.

49. The antibody or antigen-binding fragment thereof of claim **46**, wherein the first and/or second CH region is a human IgG4 CH comprising one of the following mutation(s), wherein the numbering of the constant region is as per the EU index:

(a) E233P, F234V, L235A, and/or G236del;

(b) E233P, F234V, and/or L235A;

(c) S228P and/or L235E; or

(d) S228P and/or L235A.

50. The antibody or antigen-binding fragment thereof of any one of claims **41-45**, wherein the first and/or second CH region comprise one or more mutations that enhance effector function.

51. The antibody or antigen-binding fragment thereof of claim **50**, wherein the first and/or second CH region is a human IgG1 CH comprising one of the following mutation(s), wherein the numbering of the constant region is as per the EU index:

(a) F243L, R292P, Y300L, V305I, and/or P396L;

(b) S239D and/or I332E;

(c) S239D, I332E, and/or A330L;

(d) S298A, E333A, and/or K334A;

(e) G236A, S239D, and/or I332E;

(f) K326W and/or E333S;

(g) S267E, H268F, and/or S324T; or

(h) E345R, E430G, and/or S440Y.

52. The antibody or antigen-binding fragment thereof of any one of claims **43-51**, wherein at least one of the first and second heavy chains is non-fucosylated.

53. The antibody or antigen-binding fragment thereof of claim **50**, wherein the antibody or antigen-binding fragment thereof is produced in a cell line

(a) having an alpha-1,6-fucosyltransferase (Fut8) knockout; or

(b) line overexpressing β1,4-N-acetylglucosaminyltransferase III (GnT-III) and optionally overexpressing Golgi μ- mannosidase II (ManII).

54. The antibody or antigen-binding fragment thereof of any one of claims **43-53**, wherein the first and/or second CL region is a human kappa constant region.

55. The antibody or antigen-binding fragment thereof of any one of claims **43-54**, wherein:

(a) (i) the first antigen-binding domain comprises:

a VH region comprising HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 3, 21, and 5, respectively; and

a VL region comprising LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 32, 12, and 13, respectively; and

(ii) the second antigen-binding domain comprises:

a VH region comprising HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 43, respectively; and

a VL region comprising LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 48, respectively; or

(b) (i) the first antigen-binding domain comprises:

a VH region comprising HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 122, 123, and 124, respectively; and

a VL region comprising LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 127, 128, and 129, respectively; and

(ii) the second antigen-binding domain comprises:

a VH region comprising HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 43, respectively; and

a VL region comprising LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 48, respectively.

56. The antibody or antigen-binding fragment thereof of claim **55**, wherein:

(a) the first antigen-binding domain comprises a VH region having the amino acid sequence of SEQ ID NO: 25, and a VL region having the amino acid sequence of SEQ ID NO: 38; and

the second antigen-binding domain comprises a VH region having the amino acid sequence of SEQ ID NO: 52, and a VL region having the amino acid sequence of SEQ ID NO: 87; or

(b) the first antigen-binding domain comprises a VH region having the amino acid sequence of SEQ ID NO: 121, and a VL region having the amino acid sequence of SEQ ID NO: 126; and

the second antigen-binding domain comprises a VH region having the amino acid sequence of SEQ ID NO: 52, and a VL region having the amino acid sequence of SEQ ID NO: 87.

57. The antibody or antigen-binding fragment thereof of any one of claims **1-56**, wherein the antigen-binding fragment is a Fab, a Fab', a Fab2, a F(ab')2, Fv, a single-chain Fv (scFv), or a diabody.

58. The antibody or antigen-binding fragment thereof of any one of claims **1-57**, wherein the antibody or antigen-binding fragment thereof is conjugated to an agent.

59. The antibody or antigen-binding fragment thereof of claim **58**, wherein the agent is a cytotoxic agent or label.

60. A composition comprising the antibody or antigen-binding fragment thereof of any one of claims **1-59.**

61. The composition of claim **60**, wherein the antibody or antigen-binding fragment comprises an Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, optionally wherein less than 50% of the N-glycoside-linked carbohydrate chains contain a fucose residue.

62. The composition of claim **61**, wherein substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue.

63. An antibody or antigen binding fragment thereof that is capable of binding to siglec-6, comprising:

(a) a heavy chain variable (VH) region comprising:

heavy chain complementarity-determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 133, 141, or 149, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, 142, 150, or 160, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135, 143, or 151, or a sequence differing in 1 or 2 amino acids therefrom; and

(b) a light chain variable (VL) region comprising:

light chain complementarity-determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 137, 145, 153, or 165, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, 146, or 154, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139, 147, or 155, or a sequence differing in 1 or 2 amino acids therefrom.

64. The antibody or antigen binding fragment thereof of claim **63**, comprising:

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 133, 141, or 149,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, 142, 150, or 160, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135, 143, or 151; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 137, 145, 153, or 165,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, 146, or 154, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139, 147, or 155.

65. The antibody or antigen binding fragment thereof of claim **63**, comprising:

(a) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 133, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135, or a sequence differing in 1 or 2 amino acids therefrom; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 137, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139, or a sequence differing in 1 or 2 amino acids therefrom; or

(b) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 141, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 142, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 143, or a sequence differing in 1 or 2 amino acids therefrom; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 145, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 146, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 147, or a sequence differing in 1 or 2 amino acids therefrom; or

(c) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 149, or a sequence differing in 1 or 2 amino acids therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 150 or 160, or a sequence differing in 1 or 2 amino acids therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 151, or a sequence differing in 1 or 2 amino acids therefrom; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 153 or 165, or a sequence differing in 1 or 2 amino acids therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 154, or a sequence differing in 1 or 2 amino acids therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 155, or a sequence differing in 1 or 2 amino acids therefrom.

66. The antibody or antigen binding fragment thereof of claim **65**, comprising:

(a) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 133,

HCDR2 having the amino acid sequence of SEQ ID NO: 134, and

HCDR3 having the amino acid sequence of SEQ ID NO: 135; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 137,

LCDR2 having the amino acid sequence of SEQ ID NO: 138, and

LCDR3 having the amino acid sequence of SEQ ID NO: 139; or

(b) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 141,

HCDR2 having the amino acid sequence of SEQ ID NO: 142, and

HCDR3 having the amino acid sequence of SEQ ID NO: 143; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 145,

LCDR2 having the amino acid sequence of SEQ ID NO: 146, and

LCDR3 having the amino acid sequence of SEQ ID NO: 147; or

(c) (i) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 149,

HCDR2 having the amino acid sequence of SEQ ID NO: 150 or 160, and

HCDR3 having the amino acid sequence of SEQ ID NO: 151; and

(ii) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 153 or 165,

LCDR2 having the amino acid sequence of SEQ ID NO: 154, and

LCDR3 having the amino acid sequence of SEQ ID NO: 155.

67. The antibody or antigen binding fragment thereof of any one of claims **63-66**, comprising:

(a) a VH region comprising:

(i) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 133, 134, and 135, respectively;

(ii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 141, 142, and 143, respectively;

(iii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 149, 150, and 151, respectively; or

(iv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 149, 160, and 151, respectively; and

(b) a VL region comprising:

(i) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 137, 138, and 139, respectively;

(ii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 145, 146, and 147, respectively;

(iii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 153, 154, and 155, respectively; or

(iv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 165, 154, and 155, respectively.

68. The antibody or antigen binding fragment thereof of any one of claims **63-67**, wherein the VH region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 132, 140, 148, 159, or 162; and

the VL region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 136, 144, 152, 164, 167, or 169.

69. The antibody or antigen binding fragment thereof of any one of claims **63-68**, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 132, 140, 148, 159, or 162; and

the VL region comprises the amino acid sequence of SEQ ID NO: 136, 144, 152, 164, 167, or 169.

70. The antibody or antigen-binding fragment thereof of any one of claims **63-69**, wherein

(a) the VH region comprises the amino acid sequence of SEQ ID NO: 132, and the VL region comprises the amino acid sequence of SEQ ID NO: 139;

(b) the VH region comprises the amino acid sequence of SEQ ID NO: 140, and the VL region comprises the amino acid sequence of SEQ ID NO: 144; or

(c) the VH region comprises the amino acid sequence of SEQ ID NO: 148, and the VL region comprises the amino acid sequence of SEQ ID NO: 152.

71. The antibody or antigen-binding fragment thereof of any one of claims **63-69**, wherein

(a) the VH region comprises the amino acid sequence of SEQ ID NO: 159, and the VL region comprises the amino acid sequence of SEQ ID NO: 164;

(6) the VH region comprises the amino acid sequence of SEQ ID NO: 162, and the VL region comprises the amino acid sequence of SEQ ID NO: 167; or

(c) the VH region comprises the amino acid sequence of SEQ ID NO: 162, and the VL region comprises the amino acid sequence of SEQ ID NO: 169.

72. The antibody or antigen-binding fragment thereof of any one of claims **63-71**, wherein the antibody is a humanized antibody.

73. A humanized antibody or antigen binding fragment thereof that is capable of binding to siglec-6, comprising:

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

heavy chain complementarity-determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 3,

HCDR2 having the amino acid sequence of SEQ ID NO: 21, and

HCDR3 having the amino acid sequence of SEQ ID NO: 5; and

(a) a light chain variable domain (VL) comprising:

lilght chain complementarity-determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 32,

LCDR2 having the amino acid sequence of SEQ ID NO: 12, and

LCDR3 having the amino acid sequence of SEQ ID NO: 13.

74. The antibody or antigen binding fragment thereof of claim **73**, wherein

the VH region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 20, 23, 25, 27, or 29; and

the VL region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 31, 36, or 38.

75. The antibody or antigen-binding fragment thereof of claim **73** or **74**, wherein

the VH region comprises the amino acid sequence of SEQ ID NO: 20, 23, 25, 27, or 29; and

the VL region comprisesthe amino acid sequence of SEQ ID NO: 31, 36, or 38.

76. The antibody or antigen-binding fragment thereof of any one of claims **73-75**, wherein

(a) the VH region comprises the amino acid sequence of SEQ ID NO: 23, and the VL region comprises the amino acid sequence of SEQ ID NO: 38;

(b) the VH region comprises the amino acid sequence of SEQ ID NO: 25, and the VL region comprises the amino acid sequence of SEQ ID NO: 38;

(c) the VH region comprises the amino acid sequence of SEQ ID NO: 27, and the VL region comprises the amino acid sequence of SEQ ID NO: 38; or

(d) the VH region comprises the amino acid sequence of SEQ ID NO: 29, and the VL region comprises the amino acid sequence of SEQ ID NO: 38.

77. The antibody or antigen-binding fragment thereof of claim **76**, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 25, and the VL region comprises the amino acid sequence of SEQ ID NO: 38.

78. The antibody or antigen binding fragment thereof of any one of claims **63-77**, wherein the antibody or antigen binding fragment thereof is capable of binding siglec-6 with a K_D value of at most about 10 nM, at most about 5 nM, or at most about 1 nM.

79. The antibody or antigen binding fragment thereof of any one of claims **63-78**, wherein the antibody or antigen binding fragment thereof

(a) is capable of binding human siglec-6;

(b) is capable of binding cynomolgus siglec-6;

(c) is capable of binding a mast cell, optionally preferentially than another immune cell (e.g., T cell, dendritic cell, macrophage, neutrophil, or basophil);

(d) does not induce mast cell degranulation; and/or

(e) has a comparable or lower internalization rate compared to a reference antibody capable of binding siglect-6, optionally wherein the reference antibody comprises a VH region comprising the amino acid sequence of SEQ ID NO: 2 and a VL region comprising the amino acid sequence of SEQ ID NO: 10.

80. A humanized antibody or antigen binding fragment thereof that is capable of binding to c-Kit, comprising:

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

heavy chain complementarity-determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 41, or a sequence differing in 1 amino acid therefrom,

HCDR2 having the amino acid sequence of SEQ ID NO: 53, or a sequence differing in 1 amino acid therefrom, and

HCDR3 having the amino acid sequence of SEQ ID NO: 43, or a sequence differing in 1 amino acid therefrom; and

(a) a light chain variable domain (VL) comprising:

lilght chain complementarity-determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 46, or a sequence differing in 1 amino acid therefrom,

LCDR2 having the amino acid sequence of SEQ ID NO: 47, or a sequence differing in 1 amino acid therefrom, and

LCDR3 having the amino acid sequence of SEQ ID NO: 48, or a sequence differing in 1 amino acid therefrom.

81. The antibody or antigen binding fragment thereof of claim **80**, comprising

(a) a VH region comprising:

HCDR1 having the amino acid sequence of SEQ ID NO: 41,

HCDR2 having the amino acid sequence of SEQ ID NO: 53, and

HCDR3 having the amino acid sequence of SEQ ID NO: 43; and

(b) a VL region comprising:

LCDR1 having the amino acid sequence of SEQ ID NO: 46,

LCDR2 having the amino acid sequence of SEQ ID NO: 47, and

LCDR3 having the amino acid sequence of SEQ ID NO: 48.

82. The antibody or antigen binding fragment thereof of claim **80**, wherein the VH comprises:

(a) HCDR1 having the amino acid sequence of SEQ ID NO: 60, 61, or 62,

(b) HCDR2 having the amino acid sequence of SEQ ID NO: 63, 64, 65, 66, 67, or 68, and

(c) HCDR3 having the amino acid sequence of SEQ ID NO: 69, 70, 71, or 72.

83. The antibody or antigen binding fragment thereof of claim **80** or **82**, wherein the VL comprises:

(a) LCDR1 having the amino acid sequence of SEQ ID NO: 90, 91, 92, 93, 94, or 95,

(b) LCDR2 having the amino acid sequence of SEQ ID NO: 96 or 97, and

(c) LCDR3 having the amino acid sequence of SEQ ID NO: 98, 99, 100, 101, 102, 103, or 104.

84. The antibody or antigen-binding fragment thereof of any one of claims **80-83**, wherein:

(a) the VH region comprises:

(i) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 43, respectively;

(ii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 60, 53, and 43, respectively;

(iii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 61, 53, and 43, respectively;

(iv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 62, 53, and 43, respectively;

(v) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 63, and 43, respectively;

(vi) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 64, and 43, respectively;

(vii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 65, and 43, respectively;

(viii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 66, and 43, respectively;

(ix) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 67, and 43, respectively;

(x) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 68, and 43, respectively;

(xi) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 69, respectively;

(xii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 70, respectively;

(xiii) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 71, respectively; or

(xiv) HCDR1, HCDR2, and HCDR3 having the amino acid sequence of SEQ ID NOs: 41, 53, and 72, respectively; and

(b) a VL region comprising:

(i) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 48, respectively;

(ii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 90, 47, and 48, respectively;

(iii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 91, 47, and 48, respectively;

(iv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 92, 47, and 48, respectively;

(v) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 93, 47, and 48, respectively;

(vi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 94, 47, and 48, respectively;

(vii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 95, 47, and 48, respectively;

(viii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 96, and 48, respectively;

(ix) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 97, and 48, respectively;

(x) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 98, respectively;

(xi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 99, respectively;

(xii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 100, respectively;

(xiii) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 101, respectively;

(xiv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 102, respectively;

(xv) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 103, respectively; or

(xvi) LCDR1, LCDR2, and LCDR3 having the amino acid sequence of SEQ ID NOs: 46, 47, and 104, respectively.

85. The antibody or antigen-binding fragment thereof of any one of claims **80-84**, wherein:

the VH region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 52, 55, 57, 59, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, or 85; and

the VL region comprises an amino acid sequence having at least 70%, at least 80%, at least 90%, or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 87, 89, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119.

86. The antibody or antigen-binding fragment thereof of any one of claims **80-85**, wherein:

the VH region comprises the amino acid sequence of SEQ ID NO: 52, 55, 57, 59, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, or 85; and

the VL region comprisesthe amino acid sequence of SEQ ID NO: 87, 89, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119.

87. The antibody or antigen-binding fragment thereof of any one of claims **80-86**, wherein

(a) the VH region comprises the amino acid sequence of SEQ ID NO: 40, and the VL region comprises the amino acid sequence of SEQ ID NO: 45;

(b) the VH region comprises the amino acid sequence of SEQ ID NO: 52, and the VL region comprises the amino acid sequence of SEQ ID NO: 87;

(c) the VH region comprises the amino acid sequence of SEQ ID NO: 55, and the VL region comprises the amino acid sequence of SEQ ID NO: 87;
(d) the VH region comprises the amino acid sequence of SEQ ID NO: 57, and the VL region comprises the amino acid sequence of SEQ ID NO: 87;
(e) the VH region comprises the amino acid sequence of SEQ ID NO: 59, and the VL region comprises the amino acid sequence of SEQ ID NO: 87;
(f) the VH region comprises the amino acid sequence of SEQ ID NO: 52, and the VL region comprises the amino acid sequence of SEQ ID NO: 89; or
(g) the VH region comprises the amino acid sequence of SEQ ID NO: 55, and the VL region comprises the amino acid sequence of SEQ ID NO: 89.

88. The antibody or antigen-binding fragment thereof of claim **87**, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 52, and the VL region comprises the amino acid sequence of SEQ ID NO: 87.

89. The antibody or antigen binding fragment thereof of any one of claims **80-88**, wherein the antibody or antigen binding fragment thereof is capable of binding siglec-6 with a K_D value of between about 10 and about 500 nM, between about 20 and about 100 nM; or between about 25 and about 80 nM.

90. The antibody or antigen binding fragment thereof of any one of claims **80-89**, wherein the antibody or antigen binding fragment thereof
(a) is capable of binding human c-Kit;
(b) is capable of binding cynomolgus c-Kit; and/or
(c) has a comparable or lower toxicity compared to a reference antibody capable of binding c-Kit, optionally wherein the reference antibody comprises a VH region comprising the amino acid sequence of SEQ ID NO: 40, and a VL region comprising the amino acid sequence of SEQ ID NO: 45.

91. The antibody or antigen binding fragment thereof of any one of claims **63-90**, wherein the antigen binding fragment is a Fab, a F(ab')2, a Fab', a single-chain Fv (scFv), an Fv fragment, a Fd fragment, or a diabody.

92. The antibody or antigen binding fragment thereof of any one of claims **63-91**, wherein the antibody or antigen binding fragment thereof comprises an antibody heavy chain constant region, optionally wherein the constant region is a human IgG heavy chain constant region, optionally wherein the human IgG heavy chain constant region is an IgG1 heavy chain constant region.

93. The antibody or antigen binding fragment thereof of claim **92**, wherein the constant region comprises one or more mutations that increase serum half-life of the antibody or antigen binding fragment thereof.

94. The antibody or antigen binding fragment thereof of claim **92** or **93**, wherein the constant region comprises one or more mutations that reduce or abrogate effector function.

95. The antibody or antigen binding fragment thereof of claim **92** or **93**, wherein the constant region comprises one or more mutations that enhance effector function.

96. The antibody or antigen-binding fragment thereof of any one of claims **63-95**, wherein at least one of the first and second heavy chains is afucosylated (e.g., at least 75% afucosylated).

97. The antibody or antigen binding fragment thereof of any one of claims **63-96**, conjugated to a therapeutic agent or a label.

98. A polynucleotide encoding an antibody or antigen binding fragment thereof of any one of claims **1-59** amd **63-97**.

99. An expression vector comprising the polynucleotide of claim **96**.

100. A host cell comprising the polynucleotide of claim **98** or the expression vector of claim **99**.

101. The host cell of claim **100**, wherein the host cell is a mammalian or insect cell.

102. The host cell of claim **100** or **101**, wherein the host cell (a) comprises a Fut8 knockout; and/or (b) overexpresses GnT-III and optionally ManII.

103. A pharmaceutical composition comprising an antibody or antigen binding fragment thereof of any one of claims **1-59** amd **63-97**, and a pharmaceutically acceptable carrier.

104. A method of treating a disease or disorder in a subject in need thereof, comprising administering to the subject an effective amount of an antibody or antigen binding fragment thereof of any one of claims **1-59** amd **63-97**, a composition of any one of claims **58-60**, or a pharmaceutical composition of claim **103**.

105. The method of claim **104**, wherein the disease or disorder is a mast cell associated disease or disorder.

106. The method of claim **105**, wherein the disease or disorder is an autoimmune disease, an allergy, an inflammation, pruritus, or a cancer.

107. The method of any one of claims **104-106**, wherein the disease or disorder is associated with expression of siglec-6 and/or c-Kit.

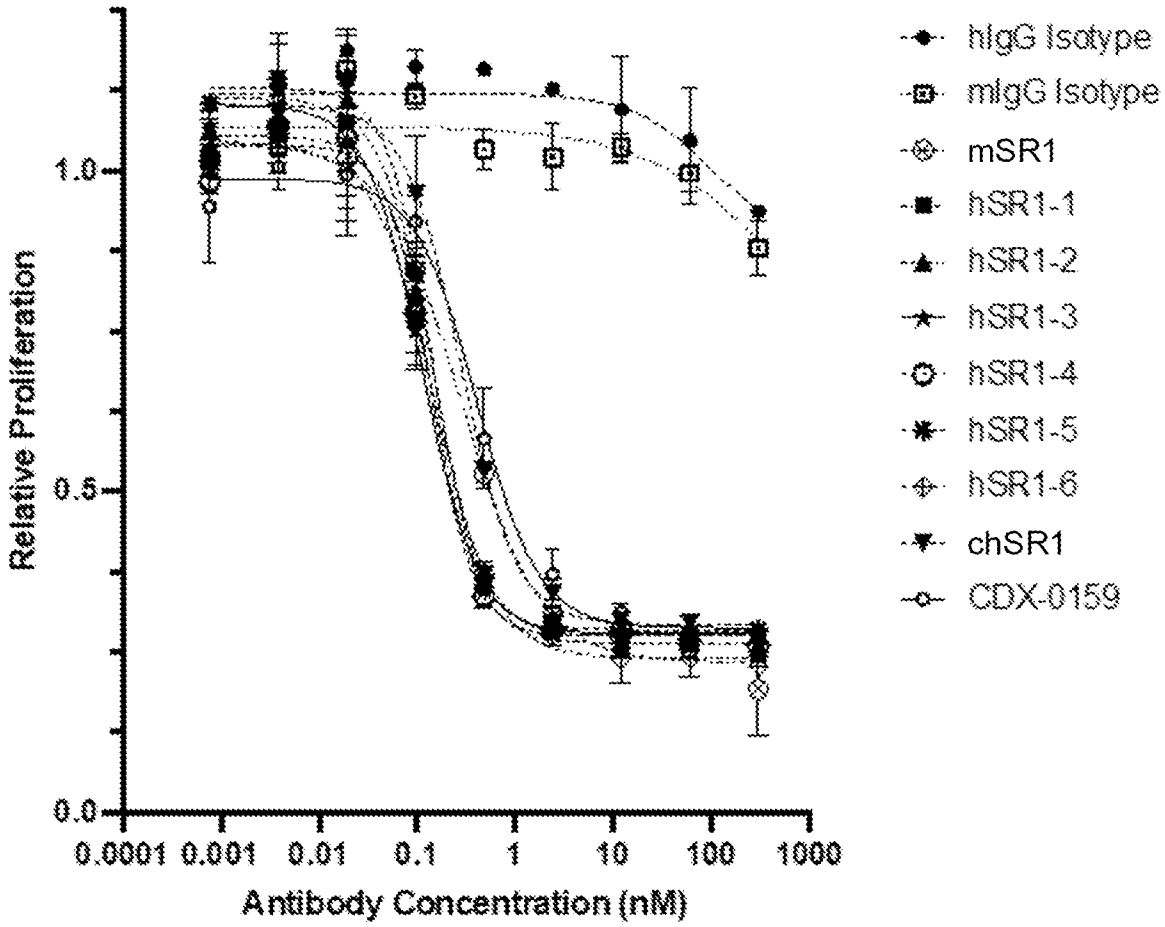
Relative Proliferation
1.0
0.5
0.0
0.0001 0.001 0.01 0.1 1 10 100 1000
Antibody Concentration (nM)
hIgG Isotype
mIgG Isotype
mSR1
hSR1-1
hSR1-2
hSR1-3
hSR1-4
hSR1-5
hSR1-6
chSR1
CDX-0159

FIG. 1

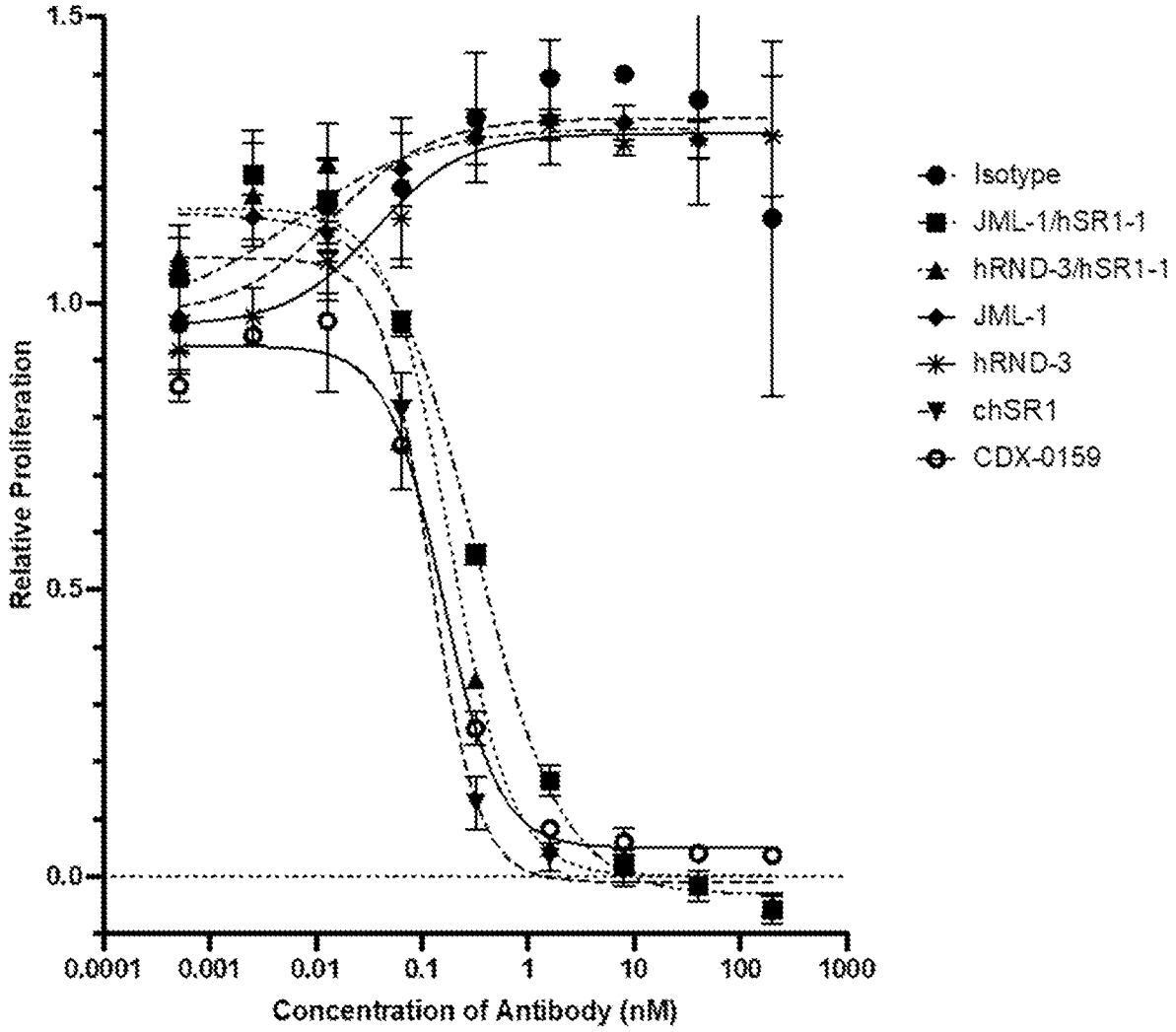
1.5
1.0
0.5
0.0
Relative Proliferation
0.0001
0.001
0.01
0.1
1
10
100
1000
Concentration of Antibody (nM)
Isotype
JML-1/hSR1-1
hRND-3/hSR1-1
JML-1
hRND-3
chSR1
CDX-0159

FIG. 2

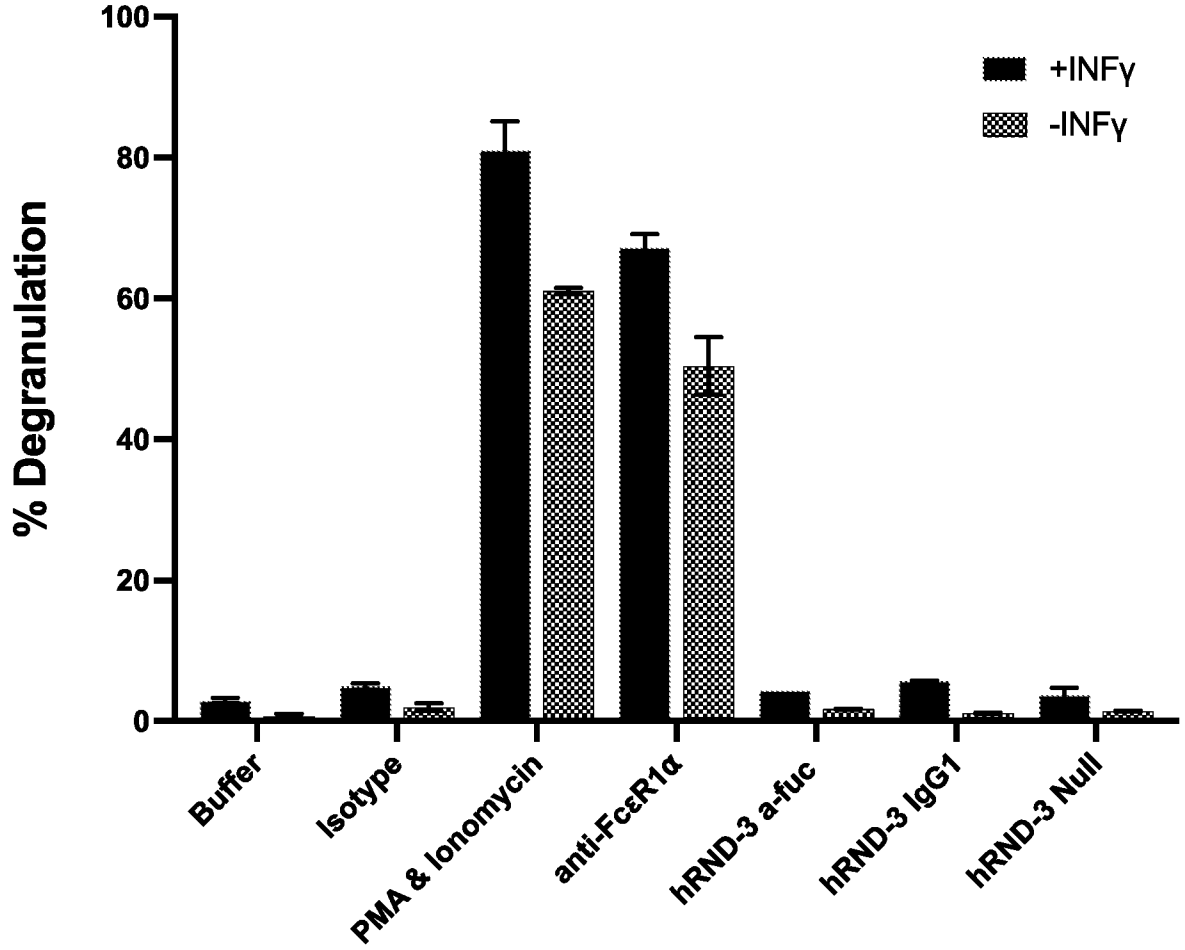
100
80
60
40
20
0
% Degranulation
+INFγ
-INFγ
Buffer
Isotype
PMA & Ionomycin
anti-FcεR1α
hRND-3 a-fuc
hRND-3 IgG1
hRND-3 Null

FIG. 3