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(54) Title: BISPECIFIC ANTIBODIES TO SIGLEC-6 AND SIGLEC-8 AND METHODS OF USE THEREOF

(57) Abstract: The present disclosure provides multispecific (e.g. bispecific) antibodies targeting Siglec-6 and Siglec-8, as well as nucleic acids, vectors, host cells, compositions, methods, uses, and kits related thereto.

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BISPECIFIC ANTIBODIES TO SIGLEC-6 AND SIGLEC-8 AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 63/471,925, filed June 8, 2023, the disclosures of which are incorporated herein by reference in their entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The content of the electronic sequence listing (701712002040seqlist.xml; Size: 287,874 bytes; and Date of Creation: June 5, 2024) is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present disclosure relates to multispecific (*e.g.*, bispecific) antibodies that bind human Siglec-6 and human Siglec-8, as well as nucleic acids, vectors, host cells, compositions, methods, uses, and kits related thereto.

BACKGROUND

[0004] Siglecs (sialic acid-binding immunoglobulin-like lectins) are single-pass transmembrane cell surface proteins found predominantly on leukocytes and that are characterized by their specificity for sialic acids attached to cell-surface glycoconjugates. The Siglec family contains at least 15 members that are found in mammals (Pillai et al., *Annu Rev Immunol.*, 2012, 30:357-392). These members include sialoadhesion (Siglec-1), CD22 (Siglec-2), CD33 (Siglec-3), myelin associated glycoprotein (Siglec-4), Siglec-5, OBBP1 (Siglec-6), AIRM1 (Siglec-7), SAF-2 (Siglec-8), and CD329 (Siglec-9).

[0005] Siglec-8, a member of the CD33-related family of sialic acid-binding, immunoglobulin-like lectins (Siglecs), is a transmembrane cell surface protein with restricted tissue distribution, expressed selectively on the surface of eosinophils, mast cells and, at lower levels, on basophils. Siglec-8 contains 3 extracellular immunoglobulin-like domains, a transmembrane region, and a cytoplasmic tail containing 2 tyrosine-based signaling motifs including an immunoreceptor tyrosine-based inhibitory motif with inhibitory function. Engagement of Siglec-8 in mast cells can result in inhibition of mediator release, and in eosinophils can induce apoptosis (Bochner, B. (2009) *Clin. Exp. Allergy* 39:317-324).

[0006] Siglec-6 (also known as CD327) is an inhibitory receptor that is selectively expressed on mast cells, *e.g.*, human tissue mast cells, HMC-1 cells, and CD34+ derived human mast cells. Mast cells are considered pathogenic drivers of numerous autoimmune and inflammatory diseases, including but not limited to food allergy, mast cell activation syndrome, mastocytosis, IPF, COPD, and others. *See, e.g.*, Yu, Y. *et al.* (2018) *Front. Immunol.* 9:2138; Yokoi, H. *et al.* (2006) *Allergy* 61:769-76; WO2005124358; and US PG Pub. Nos. US20060269556A1 and US20080267973A1. Engagement of Siglec-6 with antibody can inhibit IgE- and non-IgE-mediated mast cell activation (Robida, P.A. *et al.* (2022) *Cells* 11(7):1138; Schanin, J. *et al.* (2022) *Commun. Biol.* 5(1):1226).

[0007] There remains a need for antibodies that show potent mast cell inhibitory and/or eosinophil depletion activity. The present disclosure demonstrates that bispecific antibodies targeting Siglec-6 and Siglec-8 may lead to improved properties (including but not limited to greater mast cell inhibition, eosinophil depletion, and/or Siglec-6/-8 internalization) as compared to monospecific antibodies targeting either molecule alone.

[0008] All references cited herein, including patent applications, patent publications, and scientific literature, are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

BRIEF SUMMARY

[0009] To meet this and other needs, the present disclosure relates, *inter alia*, to multispecific (*e.g.*, bispecific) antibodies that bind to human Siglec-6 and human Siglec-8. The present disclosure demonstrates that bispecific antibodies targeting Siglec-6 and Siglec-8 can provide enhanced mast cell inhibition, eosinophil depletion, and/or Siglec-6/-8 internalization, as compared to monospecific antibodies targeting either molecule alone. Related nucleic acids, vectors, host cells, methods, uses, and kits are further provided.

[0010] Accordingly, certain aspects of the present disclosure relate to multispecific (*e.g.*, bispecific antibodies) antibodies comprising a first antigen binding domain that binds (*e.g.*, specifically binds) to human Siglec-6 and a second antigen binding domain that binds (*e.g.*, specifically binds) to human Siglec-8. In some embodiments, the first antigen binding domain comprises a first heavy chain variable (VH) region and a first light chain variable (VL) region, and the second antigen binding domain comprises a second heavy chain variable (VH) region and a second light chain variable (VL) region.

[0011] In some embodiments, the first antigen binding domain is a humanized antigen binding domain that binds to Domain 1 of an extracellular domain of human Siglec-6. In some embodiments, Domain 1 of the extracellular domain of human Siglec-6 comprises the amino acid sequence of SEQ ID NO:121. In some embodiments, the first antigen binding domain binds to Domain 2 of an extracellular domain of human Siglec-6. In some embodiments, Domain 2 of the extracellular domain of human Siglec-6 comprises the amino acid sequence of SEQ ID NO:122. In some embodiments, the first antigen binding domain binds to Domain 3 of an extracellular domain of human Siglec-6. In some embodiments, Domain 3 of the extracellular domain of human Siglec-6 comprises the amino acid sequence of SEQ ID NO:123. In some embodiments, the first antigen binding domain is a humanized antigen binding domain. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:124, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:125, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:126; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:127, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:128, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:129. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:254, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:255. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:208, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:209, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:210; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:211, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:212, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:213. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:260, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:261. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:202, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:203, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:204; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:205, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:206, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:207. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:256, and

wherein the first VL region comprises the amino acid sequence of SEQ ID NO:257. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:130, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:131, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:132; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:133, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:134, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:135. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:252, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:253. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:196, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:197, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:198; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:199, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:200, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:201. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:250, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:251. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:136, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:137, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:138; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:139, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:140, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:141. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:258, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:259. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:190, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:191, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:192; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:193, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:194, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:195. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:248, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:249. In some embodiments, the first VH region comprises an HVR-H1

comprising the amino acid sequence of SEQ ID NO:172, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:173, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:174; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:175, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:176, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:177. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:242, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:243. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:148, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:149, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:150; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:151, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:152, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:153. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:240, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:241. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:142, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:143, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:144; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:145, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:146, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:147. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:234, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:235. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:154, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:155, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:156; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:157, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:158, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:159. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:232, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:233. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:160, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:161, and an HVR-H3 comprising

the amino acid sequence of SEQ ID NO:162; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:163, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:164, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:165. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:236, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:237. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:166, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:167, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:168; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:169, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:170, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:171. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:238, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:239. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:178, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:179, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:180; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:181, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:182, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:183. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:244, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:245. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:184, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:185, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:186; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:187, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:188, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:189. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:246, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:247. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:226, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:227, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:228; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ

ID NO:229, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:230, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:231. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:266, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:267. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:214, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:215, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:216; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:217, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:218, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:219. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:262, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:263. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:220, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:221, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:222; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:223, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:224, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:225. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:264, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:265. In some embodiments, the first antigen binding domain is a human, humanized, or chimeric antigen binding domain.

[0012] In some embodiments, the second antigen binding domain is a humanized antigen binding domain with a greater binding affinity or avidity to human Siglec-8 than the binding affinity or avidity of antibody 2E2 or 2C4 to human Siglec-8. In some embodiments, the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and wherein the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66. In some embodiments, the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence selected

from SEQ ID NOs:67-70; and wherein the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:71. In some embodiments, the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:296, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:297, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and wherein the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66. In some embodiments, the second VH region comprises the amino acid sequence of SEQ ID NO:6; and the second VL region comprises the amino acid sequence of SEQ ID NO:16 or 21. In some embodiments, the second VH region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:11-14; and the second VL region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:23-24. In some embodiments, the second VH region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:2-14; and the second VL region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:16-24. In some embodiments, the second VH region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:2-10; and the second VL region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:16-22. In some embodiments, the second VH region comprises: (1) an HC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:26-29; (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61; (3) an HC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:31-36; (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62; (5) an HC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:38-43; (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and (7) an HC-FR4 comprising the amino acid sequence selected from SEQ ID NOs:45-46, and the second VL region comprises: (1) an LC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:48-49; (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64; (3) an LC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:51-53; (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65; (5) an LC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:55-58; (6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and (7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60. In some

embodiments, the second VH region comprises: (1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:26; (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61; (3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:34; (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62; (5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:38; (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and (7) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:45; and the second VL region comprises: (1) an LC-FR1 comprising the amino acid sequence of SEQ ID NO:48; (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64; (3) an LC-FR2 comprising the amino acid sequence of SEQ ID NO:51; (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65; (5) an LC-FR3 comprising the amino acid sequence of SEQ ID NO:55; (6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and (7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60. In some embodiments, the second VH region comprises: (1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:26; (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61; (3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:34; (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62; (5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:38; (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and (7) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:45; and the second VL region comprises: (1) an LC-FR1 comprising the amino acid sequence of SEQ ID NO:48; (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64; (3) an LC-FR2 comprising the amino acid sequence of SEQ ID NO:51; (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65; (5) an LC-FR3 comprising the amino acid sequence of SEQ ID NO:58; (6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and (7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60. In some embodiments, the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:88, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:91, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:94; and the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:97, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:100, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:103. In some embodiments, the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:89, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:92, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:95; and the

second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:98, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:101, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:104. In some embodiments, the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:90, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:93, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:96; and the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:99, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:102, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:105. In some embodiments, the second VH region comprises the amino acid sequence of SEQ ID NO:106; and/or the second VL region comprises the amino acid sequence of SEQ ID NO:109. In some embodiments, the second VH region comprises the amino acid sequence of SEQ ID NO:107; and/or the second VL region comprises the amino acid sequence of SEQ ID NO:110. In some embodiments, the second VH region comprises the amino acid sequence of SEQ ID NO:108; and/or the second VL region comprises the amino acid sequence of SEQ ID NO:111. In some embodiments, the second antigen binding domain is a human, humanized, or chimeric antigen binding domain.

[0013] In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:124, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:125, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:126; the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:127, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:128, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:129; the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:254, the first VL region comprises the amino acid sequence of SEQ ID NO:255, the second VH region comprises the amino acid sequence of SEQ ID NO:6, and the second VL region comprises the amino acid sequence of SEQ ID NO:16 or 21. In some embodiments, the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:208, an HVR-H2

comprising the amino acid sequence of SEQ ID NO:209, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:210; the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:211, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:212, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:213; the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66. In some embodiments, the first VH region comprises the amino acid sequence of SEQ ID NO:260, the first VL region comprises the amino acid sequence of SEQ ID NO:261, the second VH region comprises the amino acid sequence of SEQ ID NO:6, and the second VL region comprises the amino acid sequence of SEQ ID NO:16 or 21.

[0014] In some embodiments, the bispecific antibody comprises an Fc region, *e.g.*, a human IgG Fc region. In some embodiments, the Fc region is a human IgG1 Fc region. In some embodiments, the human IgG1 Fc region is non-fucosylated. In some embodiments, the Fc region is a human IgG2 Fc region. In some embodiments, the Fc region is a human IgG4 Fc region. In some embodiments, the human IgG4 Fc region comprises the amino acid substitution S228P, wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the bispecific antibody has been engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In some embodiments, the bispecific antibody comprises at least one amino acid substitution in the Fc region that improves ADCC activity. In some embodiments, at least one or two of the heavy chains of the antibody is non-fucosylated. In some embodiments, the bispecific antibody comprises a first antibody heavy chain comprising a first VH region and a first Fc region, a first antibody light chain comprising a first VL region, a second antibody heavy chain comprising a second VH region and a second Fc region, and a second antibody light chain comprising a second VL region; wherein the first VH and VL regions form the first antigen binding domain; and wherein the second VH and VL regions form the second antigen binding domain. In some embodiments, the first antibody heavy chain comprises a first constant heavy chain (CH1) region that comprises a substitution of a native cysteine to a non-cysteine amino acid and a substitution of a native non-cysteine amino acid to a cysteine amino acid; wherein the first antibody light chain comprises a constant light chain (CL)

region that comprises a substitution of a native cysteine to a non-cysteine amino acid and a substitution of a native non-cysteine amino acid to a cysteine amino acid; and wherein the substituted cysteines on the CH1 region and the CL region of the first antibody heavy and light chains, respectively, form a disulfide bond. In some embodiments, the CH1 region of the first antibody heavy chain comprises F126C and C220V substitutions, and wherein the CL region of the first antibody light chain comprises S121C and C214V substitutions, wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the second antibody heavy chain comprises a first constant heavy chain (CH1) region that comprises a substitution of a native cysteine to a non-cysteine amino acid and a substitution of a native non-cysteine amino acid to a cysteine amino acid; wherein the second antibody light chain comprises a constant light chain (CL) region that comprises a substitution of a native cysteine to a non-cysteine amino acid and a substitution of a native non-cysteine amino acid to a cysteine amino acid; and wherein the substituted cysteines on the CH1 region and the CL region of the second antibody heavy and light chains, respectively, form a disulfide bond. In some embodiments, the CH1 region of the second antibody heavy chain comprises F126C and C220V substitutions, and wherein the CL region of the second antibody light chain comprises S121C and C214V substitutions, wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the first antibody heavy chain comprises a constant light chain (CL) region that replaces a native first constant heavy chain (CH1) region, and wherein the first antibody light chain comprises a CH1 region that replaces a native CL region. In some embodiments, the second antibody heavy chain comprises a constant light chain (CL) region that replaces a native first constant heavy chain (CH1) region, and wherein the second antibody light chain comprises a CH1 region that replaces a native CL region. In some embodiments, the first antibody heavy chain comprises a CH1 region comprising substitution of one or more native, non-positively charged amino acid(s) to positively charged amino acid(s); wherein the first antibody light chain comprises a CL region comprising substitution of one or more native, non-negatively charged amino acid(s) to negatively charged amino acid(s); wherein the first VL region replaces the first VH region on the first antibody heavy chain; wherein the first VH region replaces the first VL region on the first antibody light chain; wherein the second antibody heavy chain comprises a CH1 region comprising substitution of one or more native, non-negatively charged amino acid(s) to negatively charged amino acid(s); and wherein the second antibody light chain comprises a CL region comprising substitution of one or more native, non-

positively charged amino acid(s) to positively charged amino acid(s). In some embodiments, the first antibody light chain comprises a Q124E substitution; wherein the second antibody light chain comprises E123K and Q124K substitutions; and wherein the second antibody heavy chain comprises K147E and K213E substitutions; wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the second antibody heavy chain comprises a CH1 region comprising substitution of one or more native, non-positively charged amino acid(s) to positively charged amino acid(s); wherein the second antibody light chain comprises a CL region comprising substitution of one or more native, non-negatively charged amino acid(s) to negatively charged amino acid(s); wherein the second VL region replaces the second VH region on the second antibody heavy chain; wherein the second VH region replaces the second VL region on the second antibody light chain; wherein the first antibody heavy chain comprises a CH1 region comprising substitution of one or more native, non-negatively charged amino acid(s) to negatively charged amino acid(s); and wherein the first antibody light chain comprises a CL region comprising substitution of one or more native, non-positively charged amino acid(s) to positively charged amino acid(s). In some embodiments, the second antibody light chain comprises a Q124E substitution; wherein the first antibody light chain comprises E123K and Q124K substitutions; and wherein the first antibody heavy chain comprises K147E and K213E substitutions; wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the first antibody heavy chain comprises one or more substitutions encoding a protuberance, wherein the second antibody heavy chain comprises one or more substitutions encoding a cavity, and wherein the protuberance of the first antibody heavy chain is positionable in the cavity of the second antibody heavy chain. In some embodiments, the first antibody heavy chain comprises T366W and S354C substitutions and wherein the second antibody heavy chain comprises Y349C, T366S, L368A, and Y407V substitutions, wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the second antibody heavy chain comprises one or more substitutions encoding a protuberance, wherein the first antibody heavy chain comprises one or more substitutions encoding a cavity, and wherein the protuberance of the second antibody heavy chain is positionable in the cavity of the first antibody heavy chain. In some embodiments, the second antibody heavy chain comprises T366W and S354C substitutions and wherein the first antibody heavy chain comprises Y349C, T366S, L368A, and Y407V substitutions, wherein the amino acid residues are numbered according to the EU index as in

Kabat. In some embodiments, the first antibody heavy chain comprises H435R and Y436F substitutions, wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the second antibody heavy chain comprises H435R and Y436F substitutions, wherein the amino acid residues are numbered according to the EU index as in Kabat.

[0015] In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:272, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:273 or 274, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:275, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:276 or 277. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:281, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:282 or 283, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:278, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:279 or 280. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:284, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:285 or 286, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:287, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:288 or 289. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:293, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:294 or 295, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:290, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:291 or 292. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:272, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:273, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:275, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:276. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:281, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:282, a second antibody light chain comprising the amino acid sequence of SEQ

ID NO:278, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:279. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:284, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:285, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:287, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:288. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:293, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:294, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:290, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:291. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:284, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:285 or 286, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:298, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:299 or 300. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:284, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:285, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:298, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:299.

[0016] In some embodiments, binding of the bispecific antibody to an extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to a reduced level of Siglec-6 on the cell surface. In some embodiments, binding of the bispecific antibody to an extracellular domain of human Siglec-8 when expressed on a surface of a human mast cell leads to a reduced level of Siglec-8 on the cell surface. In some embodiments, binding of the bispecific antibody to an extracellular domain of human Siglec-6 inhibits activation of a mast cell that expresses the human Siglec-6. In some embodiments, binding of the bispecific antibody to an extracellular domain of human Siglec-8 inhibits activation of a mast cell that expresses the human Siglec-8. In some embodiments, binding of the bispecific antibody to an extracellular domain of human Siglec-8 depletes eosinophils expressing human Siglec-8 *in vivo*.

[0017] In another aspect, provided herein is a composition comprising the bispecific antibody according to any one of the embodiments described herein. In some embodiments, the bispecific

antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, 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. In another aspect, provided herein is a pharmaceutical composition comprising the bispecific antibody according to any one of the embodiments described herein and a pharmaceutically acceptable carrier.

[0018] In another aspect, provided herein is a polynucleotide encoding the bispecific antibody according to any one of the embodiments described herein. In another aspect, provided herein is a kit of polynucleotides comprising a first polynucleotide encoding the first antibody heavy chain and first antibody light chain according to any one of the embodiments described herein (*e.g.*, a first antibody arm) and a second polynucleotide encoding the second antibody heavy chain and second antibody light chain according to any one of the embodiments described herein (*e.g.*, a second antibody arm). In another aspect, provided herein is a vector comprising the polynucleotide according to any one of the embodiments described herein. In another aspect, provided herein is a kit of vectors comprising a first vector encoding the first antibody heavy chain and first antibody light chain according to any one of the embodiments described herein (*e.g.*, a first antibody arm) and a second vector encoding the second antibody heavy chain and second antibody light chain according to any one of the embodiments described herein (*e.g.*, a second antibody arm).

[0019] In another aspect, provided herein is a host cell (*e.g.*, an isolated host cell) comprising the polynucleotide, vector, kit of polynucleotides, or kit of vectors according to any one of the embodiments described herein. In some embodiments, the host cell is a mammalian or insect cell. In some embodiments, the host cell is Chinese hamster ovary (CHO) cell. In some embodiments, the host cell comprises a Fut8 knockout. In some embodiments, the host cell overexpresses GnT-III. In some embodiments, the host cell additionally overexpresses ManII. In another aspect, provided herein is a method of producing a bispecific antibody, comprising culturing the host cell according to any one of the embodiments described herein under a condition that produces the bispecific antibody. In some embodiments, the method further comprises recovering the bispecific antibody produced by the host cell. In another aspect, provided herein is a bispecific antibody produced by the method according to any one of the embodiments described herein.

[0020] In another aspect, provided herein is a method of treating a disease or condition characterized by increased activity and/or number of mast cells expressing Siglec-6 and/or Siglec-8 in a subject, comprising administering to the subject an effective amount of the bispecific antibody or composition according to any one of the embodiments described herein. In another aspect, provided herein is a method of inhibiting activation of mast cells expressing Siglec-6 and/or Siglec-8 in a subject in need thereof, comprising administering to the subject an effective amount of the bispecific antibody or composition according to any one of the embodiments described herein. In another aspect, provided herein is a method of depleting mast cells expressing Siglec-6 and/or Siglec-8 in a subject in need thereof, comprising administering to the subject an effective amount of the bispecific antibody or composition according to any one of the embodiments described herein. In another aspect, provided herein is a method of treating a disease or condition mediated by cells expressing Siglec-8 in a subject (*e.g.*, in need thereof), comprising administering to the subject an effective amount of the bispecific antibody or composition according to any one of the embodiments described herein. In another aspect, provided herein is an effective amount of the bispecific antibody or composition according to any one of the embodiments described herein for use in a method of treating a disease or condition characterized by increased activity and/or number of mast cells expressing Siglec-6 and/or Siglec-8 in a subject, inhibiting activation of mast cells expressing Siglec-6 and/or Siglec-8 in a subject in need thereof, depleting mast cells expressing Siglec-6 and/or Siglec-8 in a subject in need thereof, and/or treating a disease or condition mediated by cells expressing Siglec-8 in a subject (*e.g.*, in need thereof). In another aspect, provided herein is the use of the bispecific antibody or composition according to any one of the embodiments described herein in the manufacture of a medicament for treating a disease or condition characterized by increased activity and/or number of mast cells expressing Siglec-6 and/or Siglec-8 in a subject, inhibiting activation of mast cells expressing Siglec-6 and/or Siglec-8 in a subject in need thereof, depleting mast cells expressing Siglec-6 and/or Siglec-8 in a subject in need thereof, and/or treating a disease or condition mediated by cells expressing Siglec-8 in a subject (*e.g.*, in need thereof). In some embodiments, administration of the bispecific antibody or composition results in a decrease in one or more symptoms in the subject, as compared to the one or more symptoms in the subject prior to the administration. In some embodiments, the one or more symptoms are selected from the group consisting of nausea, cramping, constipation, abdominal pain, bloating, vomiting, diarrhea, fatigue, eye pain, light sensitivity, redness, discharge, runny nose, headache,

dizziness, brain fog, itching, flushing, sweating, hives, hypotension, shortness of breath, bone pain, joint pain, weight loss, osteoporosis, angioedema, chest pain, anxiety, depression, rapid heartbeat, bronchoconstriction, and general pain. In some embodiments, the disease or condition is mastocytosis, mast cell leukemia, mast cell activation syndrome, gastroparesis, osteoporosis, osteopenia, renal osteodystrophy, bone fracture, Alzheimer's disease, chronic neuropathic pain, hyperalgesia, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), graft vs. host disease (GVH), colitis, hereditary alpha tryptasemia, neurofibroma, Kounis syndrome, urticaria, atopic dermatitis, contact dermatitis, angioedema, prurigo nodularis, cholangitis, psoriasis, irritable bowel syndrome (IBS), functional dyspepsia, asthma, allergy, keloid, chronic rhinosinusitis, aspirin exacerbated respiratory disease (AERD), chronic obstructive pulmonary disease (COPD), bullous pemphigoid, idiopathic pulmonary fibrosis, systemic sclerosis, interstitial cystitis, hidradenitis suppurativa, alopecia areata, vitiligo, mast cell gastrointestinal disease, Crohn's disease, rheumatoid arthritis, gastroesophageal reflux disease, viral infection, achalasia, postural tachycardia syndrome, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), complex regional pain syndrome, osteoarthritis, or Ehlers-Danlos syndrome. In some embodiments, the subject has or has been diagnosed with mastocytosis, mast cell leukemia, mast cell activation syndrome, gastroparesis, osteoporosis, osteopenia, renal osteodystrophy, bone fracture, Alzheimer's disease, chronic neuropathic pain, hyperalgesia, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), graft vs. host disease (GVH), colitis, hereditary alpha tryptasemia, neurofibroma, Kounis syndrome, urticaria, atopic dermatitis, contact dermatitis, angioedema, prurigo nodularis, cholangitis, psoriasis, irritable bowel syndrome (IBS), functional dyspepsia, asthma, allergy, keloid, chronic rhinosinusitis, aspirin exacerbated respiratory disease (AERD), chronic obstructive pulmonary disease (COPD), bullous pemphigoid, idiopathic pulmonary fibrosis, systemic sclerosis, interstitial cystitis, hidradenitis suppurativa, alopecia areata, vitiligo, mast cell gastrointestinal disease, Crohn's disease, rheumatoid arthritis, gastroesophageal reflux disease, viral infection, achalasia, postural tachycardia syndrome, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), complex regional pain syndrome, osteoarthritis, or Ehlers-Danlos syndrome. In some embodiments, the disease or disorder is selected from the group consisting of allergic rhinitis, nasal polyposis, pauci granulocytic asthma, acute or chronic airway hypersensitivity, eosinophilic esophagitis, eosinophilic leukemia, Churg-Strauss syndrome, inflammation associated with a cytokine, inflammation associated with cells

expressing Siglec-8, malignancy associated with cells expressing Siglec-8, physical urticaria, cold urticaria, pressure-urticaria, bullous pemphigoid, food allergy, chronic rhinosinusitis with concomitant asthma, aspirin-exacerbated respiratory disease, adult onset non-atopic asthma with sinus disease, chronic obstructive pulmonary disease, fibrotic disease, pre-fibrotic disease, mastocytosis, advanced systemic mastocytosis, indolent systemic mastocytosis (ISM), inflammatory bowel disease (IBD), eosinophilic esophagitis (EOE), eosinophilic gastritis (EG), eosinophilic gastroenteritis (EGE), eosinophilic colitis (EOC), eosinophilic duodenitis, mast cell gastritis or mast cell gastroenteritis, gastritis or gastroenteritis with elevated mast cells, irritable bowel syndrome, irritable bowel syndrome with elevated mast cells, functional gastrointestinal disease, functional dyspepsia, allergic conjunctivitis, giant papillary conjunctivitis, chronic urticaria, allergic bronchopulmonary aspergillosis (ABPA), allergic asthma, asthma with eosinophil or mast cell phenotype, eosinophilic granulomatosis with polyangiitis (EGPA), celiac disease, gastroparesis, hypereosinophilic syndrome, atopic dermatitis, anaphylaxis, angioedema, mast cell activation syndrome/disorder, and eosinophilic fasciitis. In some embodiments, the subject is a human.

[0021] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present disclosure. These and other aspects of the present disclosure will become apparent to one of skill in the art. These and other embodiments of the present disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] **FIGS. 1A-1C** show exemplary bispecific anti-Siglec-6/anti-Siglec-8 (Siglec-6/8) antibody configurations, in accordance with some embodiments. **FIG. 1A** shows a design using an alternative interchain disulfide bond in the anti-Siglec-8 arm to promote proper light chain pairing. In this example, the anti-Siglec-8 arm includes an F126C mutation in the heavy chain CH1 region and an S121C mutation in the light chain constant (CL) region to introduce the alternative interchain disulfide, a C220V mutation in the heavy chain and a C214V mutation in the light chain to remove native interchain disulfide, as well as H435R and Y436F mutations in the heavy chain. Knobs-into-holes technology was also used to promote heavy chain heterodimerization using the S354C and T366W knob mutations on the anti-Siglec-6 arm and Y349C, T366S, L368A, and Y407V hole mutations on the anti-Siglec-8 arm. **FIG. 1B** shows a

design using swapping of CH1 and CL regions to promote proper light chain pairing, along with the knobs-into-holes scheme shown in **FIG. 1A**. Left panel shows anti-Siglec-8 on left and anti-Siglec-6 on right with CH1/CL swap on anti-Siglec-6 arm; right panel shows anti-Siglec-8 on right and anti-Siglec-6 on left with CH1/CL swap on anti-Siglec-8 arm. **FIG. 1C** shows a design using swapping of VH and VL regions and introduced electrostatic pairing of CH1 and CL to promote proper light chain pairing, along with the knobs-into-holes scheme shown in **FIG. 1A**. Left panel shows anti-Siglec-8 on left and anti-Siglec-6 on right with CH1/CL swap on anti-Siglec-6 arm; right panel shows anti-Siglec-8 on right and anti-Siglec-6 on left with CH1/CL swap on anti-Siglec-8 arm. Electrostatic pairing mutations include E123K and Q124K on the light chain and K147E and K213E on the heavy chain. Designs in **FIGS. 1B & 1C** also include the mutations to introduce the alternative interchain disulfide and remove native interchain disulfide described in **FIG. 1A**.

[0023] **FIG. 2A & 2B** show that both fucosylated and afucosylated bispecific Siglec-6/8 huIgG1 antibodies induces internalization of both Siglec-6 and Siglec-8 on human tissue mast cells. Human lung mast cells were cultured with varying concentrations of the bispecific Siglec-6/8 huIgG1 (diamond), afucosylated bispecific Siglec-6/8 huIgG1 (open diamond), anti-Siglec-6 huIgG1 (triangle), anti-Siglec-8 afucosylated huIgG1 (square), or an isotype control (circle) for 18h at 37°C. Shown are Siglec-6 expression (dMFI; **FIG. 2A**) and Siglec-8 expression (dMFI; **FIG. 2B**) as a function of drug concentration (ug/mL).

[0024] **FIG. 3** shows that bispecific Siglec-6/8 huIgG1 antibody inhibits activation of human tissue mast cells. Human lung mast cells were stimulated with an anti-FcεRI (10ug/mL) in the presence of the bispecific Siglec-6/8 huIgG1 (diamond), anti-Siglec-6 huIgG1 (triangle), anti-Siglec-8 huIgG1 (square), or an isotype control (circle) at indicated concentrations for 20 min at 37°C. Shown is % CD63+ mast cells as a function of drug concentration (ug/mL).

[0025] **FIGS. 4A & 4B** show that both fucosylated and afucosylated bispecific Siglec-6/8 huIgG1 antibodies induce Siglec-8 receptor internalization and deplete mouse blood eosinophils in vivo. Siglec-6/Siglec-8 double transgenic mice (expressing human Siglec-6 and human Siglec-8) were intraperitoneally dosed with 5mg/kg of bispecific Siglec-6/8 huIgG1, afucosylated bispecific Siglec-6/8 huIgG1, anti-Siglec-6 huIgG1, anti-Siglec-8 afucosylated huIgG1, or an isotype control for 24h (as indicated). Shown are Siglec-8 expression on eosinophils (dMFI; **FIG. 4A**) and % eosinophils out of CD45+ cells (**FIG. 4B**) as a function of drug type.

[0026] **FIG. 4C** shows that both fucosylated and afucosylated bispecific Siglec-6/8 huIgG1 antibodies induce ADCC of human peripheral blood eosinophils. Human peripheral blood eosinophils were purified and cultured overnight with purified human NK cells and treated with varying concentrations of the bispecific Siglec-6/8 huIgG1 (diamond), afucosylated bispecific Siglec-6/8 huIgG1 (open diamond), anti-Siglec-8 afucosylated huIgG1 (square), or an isotype control (circle) for 18h at 37°C. Shown is the % of viable eosinophils out of the total eosinophil population as a function of drug concentration (ug/mL).

[0027] **FIGS. 5A & 5B** show that both fucosylated and afucosylated bispecific Siglec-6/8 huIgG1 antibodies internalize Siglec-6 and Siglec-8 on mouse tissue mast cells in vivo. Siglec-6/Siglec-8 double transgenic mice (expressing human Siglec-6 and human Siglec-8) were intraperitoneally dosed with 5mg/kg of bispecific Siglec-6/8 huIgG1, afucosylated bispecific Siglec-6/8 huIgG1, anti-Siglec-6 huIgG1, anti-Siglec-8 afucosylated huIgG1, or an isotype control for 24h (as indicated). Shown are Siglec-6 expression on mast cells (dMFI; **FIG. 5A**) and Siglec-8 expression on mast cells (dMFI; **FIG. 5B**) as a function of drug type.

[0028] **FIG. 6A** shows that both fucosylated and afucosylated bispecific Siglec-6/8 huIgG1 antibodies reduce tissue mast cell activation in vivo. Siglec-6/Siglec-8 double transgenic mice (expressing human Siglec-6 and human Siglec-8) were intraperitoneally dosed with 5mg/kg of bispecific Siglec-6/8 huIgG1, bispecific Siglec-6/8 huIgG1, anti-Siglec-6 huIgG1, anti-Siglec-8 afucosylated huIgG1, or an isotype control for 24h (as indicated). Shown is CD63 expression on mast cells (MFI) as a function of drug type.

[0029] **FIG. 6B** shows that both fucosylated and afucosylated bispecific Siglec-6/8 huIgG1 antibodies inhibit activation of mouse peritoneal mast cells. Siglec-6/Siglec-8 double transgenic mice (expressing human Siglec-6 and human Siglec-8) were intravenously dosed with 10mg/kg of bispecific Siglec-6/8 huIgG1 (diamond), afucosylated bispecific Siglec-6/8 huIgG1 (open diamond), anti-Siglec-6 huIgG1 (triangle), or an isotype control (circle). After 7 days, the peritoneal mast cells were harvested and stimulated with an anti-FcεRI at indicated concentrations for 20 min at 37°C. Shown is % CD63+ mast cells as a function of anti-FcεRI concentration (ng/mL).

[0030] **FIG. 7** shows that both fucosylated and afucosylated bispecific Siglec-6/8 huIgG1 antibodies induce reduction of human tissue mast cells. Human lung mast cells were cultured with monocyte-derived macrophages and treated with varying concentrations of the bispecific Siglec-6/8 huIgG1 (diamond), afucosylated bispecific Siglec-6/8 huIgG1 (open diamond), anti-

Siglec-6 huIgG1 (triangle), anti-Siglec-8 afucosylated huIgG1 (square), or an isotype control (circle) for 18h at 37°C. Shown is % mast cells out of CD45+ cells as a function of drug concentration (ug/mL).

[0031] FIGS. 8A & 8B show the interactome of membrane and intracellular proteins that interact with Siglec-6 and Siglec-8 in mast cells. **FIG. 8A** shows a schematic of the process for analysis of Siglec co-immunoprecipitated protein complexes from primary mast cells by liquid chromatography-mass spectrometry (LC-MS). **FIG. 8B** shows the results of STRING analysis of the Siglec-6 and Siglec-8 interactomes. Visual representation of the ten largest clusters of protein interactions involved in: **1.** Cytokine/FcεRI signaling **2.** Protein processing in ER **3.** Nucleocytoplasmic transport **4.** Mitochondrial translocation **5.** Ubiquitin ligase machinery **6.** Interferon-induced proteins **7.** TGFβ signaling **8.** Pyruvate dehydrogenases **9.** PPP6 phosphatase complex **10.** Pyrophosphate metabolism.

DETAILED DESCRIPTION

I. Definitions

[0032] It is to be understood that the present disclosure is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0033] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0034] It is understood that aspects and embodiments of the present disclosure include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0035] The term "antibody" includes polyclonal antibodies, monoclonal antibodies (including full length antibodies which have an immunoglobulin Fc region), antibody compositions with polyepitopic specificity, multispecific antibodies (*e.g.*, bispecific antibodies, diabodies, and single-chain molecules), as well as antibody fragments (*e.g.*, Fab, F(ab')₂, and Fv). The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein.

[0036] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called a J chain, and contains 10 antigen binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ϵ isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_{H1}). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see *e.g.*, *Basic and Clinical Immunology*, 8th Edition, Daniel P. Sties, Abba I. Terr and Tristram G. Parslow (eds), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

[0037] The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α , δ , ϵ , γ and μ , respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in the CH sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. IgG1 antibodies can exist in multiple polymorphic variants termed allotypes (reviewed in Jefferis and Lefranc 2009. *mAbs* Vol 1 Issue 4 1-7) any of which are suitable for use in the present disclosure. Common allotypic variants in human populations are those designated by the letters a, f, n, z.

[0038] An “isolated” antibody is one that has been identified, separated and/or recovered from a component of its production environment (*e.g.*, naturally or recombinantly). In some embodiments, the isolated polypeptide is free of association with all other components from its

production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In some embodiments, the polypeptide is purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated polypeptide or antibody is prepared by at least one purification step.

[0039] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. In some embodiments, monoclonal antibodies have a C-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the C-terminus of heavy chain and/or light chain. In some embodiments, the C-terminal cleavage removes a C-terminal lysine from the heavy chain. In some embodiments, monoclonal antibodies have an N-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the N-terminus of heavy chain and/or light chain. In some embodiments, monoclonal antibodies are highly specific, being directed against a single antigenic site. In some embodiments, monoclonal antibodies are highly specific, being directed against multiple antigenic sites (such as a bispecific antibody or a multispecific antibody). The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by a variety of techniques, including, for example, the hybridoma method, recombinant DNA methods, phage-display technologies, and technologies for producing human or human-like antibodies in animals that

have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences.

[0040] The term “naked antibody” refers to an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

[0041] The terms “full-length antibody,” “intact antibody” or “whole antibody” are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0042] An “antibody fragment” comprises a portion of an intact antibody, the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂ and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0043] Papain digestion of antibodies produced 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 L chain along with the variable region domain of the H 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. 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 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 is the designation herein for 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 which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0044] The Fc fragment comprises the carboxy-terminal portions of both H 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.

[0045] “Fv” is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0046] “Single-chain Fv” also abbreviated as “sFv” or “scFv” are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. In some embodiments, the sFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0047] “Functional fragments” of the antibodies of the present disclosure comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the Fv region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0048] The monoclonal antibodies herein specifically include “chimeric” antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is (are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with an antigen of interest. As used herein, “humanized antibody” is used as a subset of “chimeric antibodies.”

[0049] “Humanized” forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, *etc.* In some embodiments, the number of these amino acid substitutions in the FR are no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409. In some embodiments, humanized antibodies are directed against a single antigenic site. In some embodiments, humanized antibodies are directed against multiple antigenic sites. An alternative humanization method is described in U.S. Pat. No. 7,981,843 and U.S. Patent Application Publication No. 2006/0134098.

[0050] The “variable region” or “variable domain” of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as “VH” and “VL”, respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

[0051] The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody-variable domain that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.*, Xu *et al.* *Immunity* 13:37-45 (2000); Johnson and Wu in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003)). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, *e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993) and Sheriff *et al.*, *Nature Struct. Biol.* 3:733-736 (1996).

[0052] A number of HVR delineations are in use and are encompassed herein. The HVRs that are Kabat complementarity-determining regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institute of Health, Bethesda, MD (1991)). Chothia HVRs refer instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The “contact” HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

<u>Loop</u>	<u>Kabat</u>	<u>Chothia</u>	<u>Contact</u>
L1	L24-L34	L26-L34	L30-L36
L2	L50-L56	L50-L56	L46-L55
L3	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H32	H30-H35B (Kabat Numbering)
H1	H31-H35	H26-H32	H30-H35 (Chothia Numbering)
H2	H50-H65	H53-H56	H47-H58
H3	H95-H102	H95-H102	H93-H101

[0053] Unless otherwise indicated, the variable-domain residues (HVR residues and framework region residues) are numbered according to Kabat *et al.*, *supra*.

[0054] “Framework” or “FR” residues are those variable-domain residues other than the HVR residues as herein defined.

[0055] The expression “variable-domain residue-numbering as in Kabat” or “amino-acid-position numbering as in Kabat,” and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat *et al.*, *supra*. Using this numbering system, the actual linear amino acid sequence may

contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, *etc.* according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a “standard” Kabat numbered sequence.

[0056] An “acceptor human framework” for the purposes herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less.

[0057] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (*e.g.*, Kabat *et al.*, *Sequences of Immunological Interest*. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The “EU numbering system” or “EU index” is generally used when referring to a residue in an immunoglobulin heavy chain constant region (*e.g.*, the EU index reported in Kabat *et al.*, *supra*). The “EU index as in Kabat” refers to the residue numbering of the human IgG1 EU antibody.

[0058] “Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, the % amino acid sequence identity of a given amino acid sequence A to, with, or

against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

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

[0059] An antibody that “binds to”, “specifically binds to” or is “specific for” a particular a polypeptide or an epitope on a particular polypeptide is one that binds to that particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or polypeptide epitope. In some embodiments, binding of a bispecific antibody described herein (*e.g.*, an antibody that binds to human Siglec-8 and human Siglec-6) to an unrelated non-Siglec-8 or -Siglec-6 polypeptide is less than about 10% of the antibody binding to Siglec-8 and/or Siglec-6 as measured by methods known in the art (*e.g.*, enzyme-linked immunosorbent assay (ELISA)). In some embodiments, a bispecific antibody that binds to a Siglec-8 and Siglec-6 has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 2\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.7\text{ nM}$, $\leq 0.6\text{ nM}$, $\leq 0.5\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (*e.g.* 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M , *e.g.*, from 10^{-9} M to 10^{-13} M) for one or both of Siglec-6 and Siglec-8.

[0060] The term “anti-Siglec-8 antibody” or “an antibody that binds to human Siglec-8” refers to an antibody that binds to a polypeptide or an epitope of human Siglec-8 without substantially binding to any other polypeptide or epitope of an unrelated non-Siglec-8 polypeptide.

[0061] The term “Siglec-8” as used herein refers to a human Siglec-8 protein. The term also includes naturally occurring variants of Siglec-8, including splice variants or allelic variants. The amino acid sequence of an exemplary human Siglec-8 is shown in SEQ ID NO:72. The amino acid sequence of another exemplary human Siglec-8 is shown in SEQ ID NO:73. In some embodiments, a human Siglec-8 protein comprises the human Siglec-8 extracellular domain fused to an immunoglobulin Fc region. The amino acid sequence of an exemplary human Siglec-8 extracellular domain fused to an immunoglobulin Fc region is shown in SEQ ID NO:74. The

amino acid sequence underlined in SEQ ID NO:74 indicates the Fc region of the Siglec-8 Fc fusion protein amino acid sequence.

Human Siglec-8 Amino Acid Sequence

GYLLQVQELVTVQEGLCVHVPCSFSPQDGWTDSDPVHGYWFRAGDRPYQDAPVATN
 NPDREVQAETQGRFQLLGDIWSNDCSLSIRDARKRDKGSYFFRLERGSMKWSYKSQLN
 YKTKQLSVFVTALTHRPDILILGTLESGHSRNLTCVWPWACKQGTTPPMISWIGASVSSPG
 PTTARSSVLTLTPKPQDHGTS LTCQVTLP GTGVTTTSTVRLDVSYPWNLTMTVFQGDA
 TASTALGNGSSLSVLEGQSLRLVCAVNSNPPARLSWTRGSLTLCPSRSSNPGLLELPRVH
 VRDEGEFTCRAQNAQGSQHISLSLSLQNEG TGTSRPVSQVTLAAVGGAGATALAFLSFC
 IIFIIVRSCRKKSARPAAGVGDTGMEDAKAIRGSASQGPLETSWKDGNPLKKPPPAVAPS
 SGEEGELHYATLSFHKVKPQDPQGQEATDSEYSEIKIHKRETAETQACLRNHNPPSSKEV
 RG (SEQ ID NO:72)

Human Siglec-8 Amino Acid Sequence

GYLLQVQELVTVQEGLCVHVPCSFSPQDGWTDSDPVHGYWFRAGDRPYQDAPVATN
 NPDREVQAETQGRFQLLGDIWSNDCSLSIRDARKRDKGSYFFRLERGSMKWSYKSQLN
 YKTKQLSVFVTALTHRPDILILGTLESGHPRNLTCVWPWACKQGTTPPMISWIGASVSSPG
 PTTARSSVLTLTPKPQDHGTS LTCQVTLP GTGVTTTSTVRLDVSYPWNLTMTVFQGDA
 TASTALGNGSSLSVLEGQSLRLVCAVNSNPPARLSWTRGSLTLCPSRSSNPGLLELPRVH
 VRDEGEFTCRAQNAQGSQHISLSLSLQNEG TGTSRPVSQVTLAAVGGAGATALAFLSFC
 IIFIIVRSCRKKSARPAAGVGDTGMEDAKAIRGSASQGPLETSWKDGNPLKKPPPAVAPS
 SGEEGELHYATLSFHKVKPQDPQGQEATDSEYSEIKIHKRETAETQACLRNHNPPSSKEV
 RG (SEQ ID NO:73)

Siglec-8 Fc Fusion Protein Amino Acid Sequence

GYLLQVQELVTVQEGLCVHVPCSFSPQDGWTDSDPVHGYWFRAGDRPYQDAPVATN
 NPDREVQAETQGRFQLLGDIWSNDCSLSIRDARKRDKGSYFFRLERGSMKWSYKSQLN
 YKTKQLSVFVTALTHRPDILILGTLESGHSRNLTCVWPWACKQGTTPPMISWIGASVSSPG
 PTTARSSVLTLTPKPQDHGTS LTCQVTLP GTGVTTTSTVRLDVSYPWNLTMTVFQGDA
 TASTALGNGSSLSVLEGQSLRLVCAVNSNPPARLSWTRGSLTLCPSRSSNPGLLELPRVH
 VRDEGEFTCRAQNAQGSQHISLSLSLQNEG TGTSRPVSQVTLAAVGGIEGRSDKTHTCPP

CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:74)

[0062] The term “Siglec-6” as used herein refers to a human Siglec-6 protein. The term also includes naturally occurring variants of Siglec-6, including splice variants or allelic variants. Siglec-6, the sialic acid binding Ig-like lectin 6, is also known as CD327, CD33L, OBBP1, CD33L1, CD33L2, and CDW327. In some embodiments, a human Siglec-6 protein is any protein or polypeptide expressed by a human *SIGLEC6* gene. An exemplary human *SIGLEC6* gene is described by NCBI Ref. Seq. Gene ID No. 946. Amino acid sequences of exemplary human Siglec-6 proteins and domains thereof are described herein. For example, in some embodiments, a human Siglec-6 protein comprises an extracellular domain (ECD) comprising the amino acid sequence

QERRFQLEGPESLTVQEGLCVLVPCRLPTTLPASYYGYGYWFLEGADVPVATNDPDEE
 VQEETRGRFHLLWDPRRKNCSLSIRDARRRDNAAYFFRLKSKWMKYGYTSSKLSVRV
 MALTHRPNISIPGTLESGHPSNLTCSPWVCEQGTPPIFSWMSAAPTSLGPRTTQSSVLT
 TPRPQDHSTNLTCQVTFPGAGVTMERTIQLNVSYAPQKVAISIFQGNAAFKILQNTSSLP
 VLEGQALRLLCDADGNPPAHL SWFQGFPALNATPISNTGVLELPQVGSAAEGDFTCRAQ
 HPLGSLQISLSLFVHWKPEGRAGGV (SEQ ID NO:120).

[0063] The term “anti-Siglec-6 antibody” or “an antibody that binds to human Siglec-6” refers to an antibody that binds to a polypeptide or an epitope of human Siglec-6 without substantially binding to any other polypeptide or epitope of an unrelated non-Siglec-6 polypeptide.

[0064] Antibodies that “induce apoptosis” or are “apoptotic” are those that induce programmed cell death as determined by standard apoptosis assays, such as binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). For example, the apoptotic activity of the bispecific antibodies (*e.g.*, an antibody that binds to human Siglec-8 and human Siglec-6) of the present disclosure can be shown by staining cells with annexin V.

[0065] Antibody “effector functions” refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q

binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptors); and B cell activation.

[0066] “Antibody-dependent cell-mediated cytotoxicity” or “ADCC” refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.*, natural killer (NK) cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies “arm” the cytotoxic cells and are required for killing of the target cell by this mechanism. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991). In some embodiments, an antibody (*e.g.*, a bispecific antibody that binds to human Siglec-8 and Siglec-6) described herein enhances ADCC. To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al.*, *PNAS USA* 95:652-656 (1998). Other Fc variants that alter ADCC activity and other antibody properties include those disclosed by Ghetie *et al.*, *Nat Biotech.* 15:637-40, 1997; Duncan *et al.*, *Nature* 332:563-564, 1988; Lund *et al.*, *J. Immunol* 147:2657-2662, 1991; Lund *et al.*, *Mol Immunol* 29:53-59, 1992; Alegre *et al.*, *Transplantation* 57:1537-1543, 1994; Hutchins *et al.*, *Proc Natl. Acad Sci USA* 92:11980-11984, 1995; Jefferis *et al.*, *Immunol Lett.* 44:111-117, 1995; Lund *et al.*, *FASEB J* 9:115-119, 1995; Jefferis *et al.*, *Immunol Lett* 54:101-104, 1996; Lund *et al.*, *J Immunol* 157:4963-4969, 1996; Armour *et al.*, *Eur J Immunol* 29:2613-2624, 1999; Idusogie *et al.*, *J Immunol* 164:4178-4184, 2000; Reddy *et al.*, *J Immunol* 164:1925-1933, 2000; Xu *et al.*, *Cell Immunol* 200:16-26, 2000; Idusogie *et al.*, *J Immunol* 166:2571-2575, 2001; Shields *et al.*, *J Biol Chem* 276:6591-6604, 2001; Jefferis *et al.*, *Immunol Lett* 82:57-65, 2002; Presta *et al.*, *Biochem Soc Trans* 30:487-490, 2002; Lazar *et al.*, *Proc. Natl. Acad. Sci. USA* 103:4005-4010, 2006; U.S. Pat. Nos. 5,624,821; 5,885,573; 5,677,425; 6,165,745; 6,277,375; 5,869,046; 6,121,022; 5,624,821; 5,648,260; 6,194,551; 6,737,056; 6,821,505; 6,277,375; 7,335,742; and 7,317,091.

[0067] The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. Suitable native-sequence Fc regions for use in the antibodies of the present disclosure include human IgG1, IgG2, IgG3 and IgG4. A single amino acid substitution (S228P according to Kabat numbering; designated IgG4Pro) may be introduced to abolish the heterogeneity observed in recombinant IgG4 antibody. *See* Angal, S. et al. (1993) Mol Immunol 30, 105–108.

[0068] “Non-fucosylated” 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, an antibody with reduced fucose or lacking fucose has improved ADCC function. 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, a non-fucosylated or fucose-deficient antibody composition contemplated herein is a composition wherein less than about 50% of the N-linked glycans attached to the Fc region of the antibodies in the composition comprise fucose.

[0069] The terms “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 (1,6)-linked fucose at the innermost N-acetylglucosamine (GlcNAc) residue in one or both of the N-linked oligosaccharides attached to the antibody Fc region, e.g. at position Asn 297 of the human IgG1 Fc domain (EU numbering of Fc region residues). Asn297 may also be located about + 3 amino acids upstream or downstream of position 297, i.e. between positions 294 and 300, due to minor sequence variations in immunoglobulins.

[0070] The “degree of fucosylation” is the percentage of fucosylated oligosaccharides relative to all oligosaccharides 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%. Accordingly, an individual antibody in such a composition typically comprises fucose residues

in each of the two N-linked oligosaccharides in the Fc region. Conversely, in a composition of a "fully non-fucosylated" antibody essentially none of the oligosaccharides are fucosylated, and an individual antibody in such a composition does not contain fucose residues in either of the two N-linked oligosaccharides in the Fc region. 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 can 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%).

[0071] "Binding affinity" as used herein refers to the strength of the non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). In some embodiments, the binding affinity of an antibody for a Siglec-8 or Siglec-6 polypeptide (which may be a dimer, such as an Fc fusion protein) can generally be represented by a dissociation constant (*K_d*). Affinity can be measured by common methods known in the art, including those described herein.

[0072] "Binding avidity" as used herein refers to the binding strength of multiple binding sites of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen).

[0073] An "isolated" nucleic acid molecule encoding the antibodies herein is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. In some embodiments, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies herein existing naturally in cells.

[0074] The term "pharmaceutical formulation" refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and that contains no

additional components that are unacceptably toxic to an individual to which the formulation would be administered. Such formulations are sterile.

[0075] “Carriers” as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0076] As used herein, the term “treatment” or “treating” refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. An individual is successfully “treated”, for example, if one or more symptoms associated with a disease (*e.g.*, mast cell gastritis, mast cell esophagitis, mast cell colitis, mast cell enteritis, and/or mast cell gastroenteritis) are mitigated or eliminated. For example, an individual is successfully “treated” if treatment results in increasing the quality of life of those suffering from a disease, decreasing the dose of other medications required for treating the disease, reducing the frequency of recurrence of the disease, lessening severity of the disease, delaying the development or progression of the disease, and/or prolonging survival of individuals.

[0077] As used herein, “in conjunction with” or “in combination with” refers to administration of one treatment modality in addition to another treatment modality. As such, “in conjunction with” or “in combination with” refers to administration of one treatment modality before, during or after administration of the other treatment modality to the individual.

[0078] As used herein, the term “prevention” or “preventing” includes providing prophylaxis with respect to occurrence or recurrence of a disease in an individual. An individual may be predisposed to a disease, susceptible to a disease, or at risk of developing a disease, but has not yet been diagnosed with the disease. In some embodiments, bispecific antibodies (*e.g.*, an

antibody that binds to human Siglec-8 and human Siglec-6) described herein are used to delay development of a disease or disorder of the present disclosure.

[0079] As used herein, an individual “at risk” of developing a disease (*e.g.*, eosinophilic esophagitis) may or may not have detectable disease or symptoms of disease, and may or may not have displayed detectable disease or symptoms of disease prior to the treatment methods described herein. “At risk” denotes that an individual has one or more risk factors, which are measurable parameters that correlate with development of the disease (*e.g.*, eosinophilic esophagitis), as known in the art. An individual having one or more of these risk factors has a higher probability of developing the disease than an individual without one or more of these risk factors.

[0080] An “effective amount” refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired or indicated effect, including a therapeutic or prophylactic result. An effective amount can be provided in one or more administrations. A “therapeutically effective amount” is at least the minimum concentration required to effect a measurable improvement of a particular disease. A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. A therapeutically effective amount may also be one in which any toxic or detrimental effects of the antibody are outweighed by the therapeutically beneficial effects. A “prophylactically effective amount” refers to an amount effective, at the dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in individuals prior to or at the earlier stage of disease, the prophylactically effective amount can be less than the therapeutically effective amount.

[0081] “Chronic” administration refers to administration of the medicament(s) in a continuous as opposed to acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. “Intermittent” administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

[0082] The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0083] As used herein, an “individual” or a “subject” is a mammal. A “mammal” for purposes of treatment includes humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, *etc.* In some embodiments, the individual or subject is a human.

II. Bispecific antibodies

[0084] Certain aspects of the present disclosure relate to multispecific antibodies that bind (*e.g.*, specifically bind) human Siglec-6 and human Siglec-8. In some embodiments, the multispecific (*e.g.*, bispecific) antibodies comprise one or more antigen binding domain(s) that bind to human Siglec-6 and one or more antigen binding domain(s) that bind to human Siglec-8. In some embodiments, provided herein are bispecific antibodies that comprise an antigen binding domain that binds to human Siglec-6 and an antigen binding domain that binds to human Siglec-8. Any of the exemplary antigen binding domains that bind to human Siglec-6 or human Siglec-8 described *infra* may find use in a bispecific and/or multispecific antibody of the present disclosure.

Anti-Siglec-8 antigen binding domains

[0085] Certain aspects of the present disclosure provide antigen binding domains that bind to a human Siglec-8. In some embodiments, an anti-Siglec-8 antigen binding domain (or bispecific antibody comprising said antigen binding domain) described herein has one or more of the following characteristics: (1) binds a human Siglec-8; (2) binds to an extracellular domain of a human Siglec-8; (3) binds a human Siglec-8 with a higher affinity than mouse antibody 2E2 and/or mouse antibody 2C4; (4) binds a human Siglec-8 with a higher avidity than mouse antibody 2E2 and/or mouse antibody 2C4; (5) has a T_m of about 70°C-72°C or higher in a thermal shift assay; (6) has a reduced degree of fucosylation or is non-fucosylated; (7) binds a human Siglec-8 expressed on eosinophils and induces apoptosis of eosinophils; (8) binds a human Siglec-8 expressed on mast cells and depletes or reduces the number of mast cells; (9) binds a human Siglec-8 expressed on mast cells and inhibits FcεRI-dependent activities of mast cells (*e.g.*, histamine release, PGD₂ release, Ca²⁺ flux, and/or β-hexosaminidase release, *etc.*); (10) has been engineered to improve ADCC activity; (11) binds a human Siglec-8 expressed on mast cells and kills mast cells by ADCC activity (*in vitro*, and/or *in vivo*); (12) binds to Siglec-8 of a human and a non-human primate; (13) binds to Domain 1, Domain 2, and/or Domain 3 of

human Siglec-8, or binds a Siglec-8 polypeptide comprising Domain 1, Domain 2, and/or Domain 3 of human Siglec-8 (e.g., fusion proteins described herein); and (14) depletes activated eosinophils with an EC₅₀ less than the EC₅₀ of mouse antibody 2E2 or 2C4. Any of the antigen binding domains from the antibodies described in U.S. Pat. No. 9,546,215 and/or WO2015089117 may find use in the bispecific antibodies provided herein.

[0086] In some embodiments, the human Siglec-8 comprises an amino acid sequence of SEQ ID NO:72. In some embodiments, the human Siglec-8 comprises an amino acid sequence of SEQ ID NO:73. In some embodiments, a bispecific antibody described herein binds to a human Siglec-8 expressed on mast cells and depletes or reduces the number of mast cells. In some embodiments, a bispecific antibody described herein binds to a human Siglec-8 expressed on mast cells and inhibits mast cell-mediated activity.

[0087] In one aspect, the invention provides antigen binding domains that bind to a human Siglec-8. In some embodiments, the human Siglec-8 comprises an amino acid sequence of SEQ ID NO:72. In some embodiments, the human Siglec-8 comprises an amino acid sequence of SEQ ID NO:73. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to an epitope in Domain 1 of human Siglec-8, wherein Domain 1 comprises the amino acid sequence of SEQ ID NO: 112. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to an epitope in Domain 2 of human Siglec-8, wherein Domain 2 comprises the amino acid sequence of SEQ ID NO: 113. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to an epitope in Domain 3 of human Siglec-8, wherein Domain 3 comprises the amino acid sequence of SEQ ID NO: 114. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to a fusion protein comprising the amino acid of SEQ ID NO:116 but not to a fusion protein comprising the amino acid of SEQ ID NO:115. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to a fusion protein comprising the amino acid of SEQ ID NO:117 but not to a fusion protein comprising the amino acid of SEQ ID NO:115. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to a fusion protein comprising the amino acid of SEQ ID NO:117 but not to a fusion protein comprising the amino acid of SEQ ID NO:116. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to a linear epitope in the extracellular domain of human Siglec-8. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to a conformational epitope in the extracellular domain of human Siglec-8. In some embodiments, a

bispecific antibody described herein binds to a human Siglec-8 expressed on eosinophils and induces apoptosis of eosinophils. In some embodiments, a bispecific antibody described herein binds to a human Siglec-8 expressed on mast cells and depletes mast cells. In some embodiments, a bispecific antibody described herein binds to a human Siglec-8 expressed on mast cells and inhibits mast cell-mediated activity. In some embodiments, a bispecific antibody described herein binds to a human Siglec-8 expressed on mast cells and kills mast cells by ADCC activity. In some embodiments, a bispecific antibody described herein depletes mast cells and inhibits mast cell activation. In some embodiments, a bispecific antibody herein depletes activated eosinophils and inhibits mast cell activation. In some embodiments, a bispecific antibody herein (*e.g.*, a non-fucosylated bispecific antibody) depletes blood eosinophils and inhibits mast cell activation. In some embodiments, an antibody herein (*e.g.*, a non-fucosylated bispecific antibody) depletes eosinophils from the peripheral blood and inhibits mast cell activation.

[0088] Provided herein is an anti-Siglec-8 antigen binding domain that binds to human Siglec-8 and non-human primate Siglec-8. Identification of antigen binding domains with primate cross-reactivity would be useful for preclinical testing in non-human primates. In one aspect, the invention provides anti-Siglec-8 antigen binding domains that bind to a non-human primate Siglec-8. In one aspect, the invention provides anti-Siglec-8 antigen binding domains that bind to a human Siglec-8 and a non-human primate Siglec-8. In some embodiments, the non-human primate Siglec-8 comprises an amino acid sequence of SEQ ID NO:118 or a portion thereof. In some embodiments, the non-human primate Siglec-8 comprises an amino acid sequence of SEQ ID NO:119 or a portion thereof. In some embodiments, the non-human primate is a baboon (*e.g.*, *Papio Anubis*). In some embodiments, the anti-Siglec-8 antigen binding domain that binds to a human Siglec-8 and a non-human primate Siglec-8, binds to an epitope in Domain 1 of human Siglec-8. In a further embodiment, Domain 1 of human Siglec-8 comprises the amino acid sequence of SEQ ID NO:112. In some embodiments, the anti-Siglec-8 antigen binding domain that binds to a human Siglec-8 and a non-human primate Siglec-8, binds to an epitope in Domain 3 of human Siglec-8. In a further embodiment, Domain 3 of human Siglec-8 comprises the amino acid sequence of SEQ ID NO:114. In some embodiments, the anti-Siglec-8 antigen binding domain that binds to a human Siglec-8 and a non-human primate Siglec-8 is a humanized, chimeric, or a human antigen binding domain. In some embodiments, the anti-

Siglec-8 antigen binding domain that binds to a human Siglec-8 and a non-human primate Siglec-8 is a murine antibody.

[0089] In one aspect, anti-Siglec-8 antigen binding domains that compete with murine 2E2 antibody and murine 2C4 antibody binding to Siglec-8 are provided. Anti-Siglec-8 antigen binding domains that bind to the same epitope as murine 2E2 antibody and murine 2C4 antibody are also provided. Murine antibodies to Siglec-8, 2E2 and 2C4 antibody are described in U.S. Pat. No. 8,207,305; U.S. Pat. No. 8,197,811, U.S. Pat. No. 7,871,612, and U.S. Pat. No. 7,557,191.

[0090] In one aspect, anti-Siglec-8 antigen binding domains that compete with any anti-Siglec-8 antibody described herein (*e.g.*, HEKA, HEKF, 1C3, 1H10, 4F11, 2C4, 2E2) for binding to Siglec-8 are provided. Anti-Siglec-8 antigen binding domain that bind to the same epitope as any anti-Siglec-8 antibody described herein (*e.g.*, HEKA, HEKF, 1C3, 1H10, 4F11, 2C4, 2E2) are also provided.

[0091] In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 2C4. In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 2E2. In some embodiments, the HVR is a Kabat CDR or a Chothia CDR.

[0092] In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 1C3. In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 4F11. In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 1H10. In some embodiments, the HVR is a Kabat CDR or a Chothia CDR.

[0093] In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to an epitope in Domain 1 of human Siglec-8, wherein Domain 1 comprises the amino acid sequence of SEQ ID NO:112. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to an epitope in Domain 2 of human Siglec-8, wherein Domain 2 comprises the amino acid sequence of SEQ ID NO:113. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to an epitope in Domain 3 of human Siglec-8, wherein Domain 3 comprises the amino acid sequence of SEQ ID NO:114.

[0094] In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to a fusion protein comprising the amino acid of SEQ ID NO:116 but not to a fusion protein comprising the amino acid of SEQ ID NO:115. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to a fusion protein comprising the amino acid of SEQ ID NO:117 but not to a fusion protein comprising the amino acid of SEQ ID NO:115. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to a fusion protein comprising the amino acid of SEQ ID NO:117 but not to a fusion protein comprising the amino acid of SEQ ID NO:116.

[0095] In another aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:88, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:91, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:94; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:97, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:100, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:103. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to an epitope in Domain 2 of human Siglec-8, wherein Domain 2 comprises the amino acid sequence of SEQ ID NO: 113.

[0096] In another aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:89, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:92, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:95; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:98, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:101, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:104. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to an epitope in Domain 3 of human Siglec-8, wherein Domain 3 comprises the amino acid sequence of SEQ ID NO: 114. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to human Siglec-8 and non-human primate Siglec-8.

[0097] In another aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable region and a light chain variable region, wherein the heavy

chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:90, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:93, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:96; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:99, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:102, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:105. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to an epitope in Domain 1 of human Siglec-8, wherein Domain 1 comprises the amino acid sequence of SEQ ID NO: 112. In some embodiments, the anti-Siglec-8 antigen binding domain described herein binds to human Siglec-8 and non-human primate Siglec-8.

[0098] In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66.

[0099] In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence selected from SEQ ID NOs:67-70; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66.

[0100] In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2

comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:71.

[0101] In another aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence selected from SEQ ID NOs:67-70; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:71.

[0102] In another aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:88, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:91, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:94; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:97, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:100, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:103.

[0103] In another aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:89, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:92, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:95; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:98, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:101, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:104.

[0104] In another aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:90, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:93, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:96; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:99, (ii) HVR-L2

comprising the amino acid sequence of SEQ ID NO:102, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:105.

[0105] An anti-Siglec-8 antigen binding domain described herein may comprise any suitable framework variable domain sequence, provided that the antibody retains the ability to bind human Siglec-8. As used herein, heavy chain framework regions are designated "HC-FR1-FR4," and light chain framework regions are designated "LC-FR1-FR4." In some embodiments, the anti-Siglec-8 antigen binding domain comprises a heavy chain variable domain framework sequence of SEQ ID NO:26, 34, 38, and 45 (HC-FR1, HC-FR2, HC-FR3, and HC-FR4, respectively). In some embodiments, the anti-Siglec-8 antigen binding domain comprises a light chain variable domain framework sequence of SEQ ID NO:48, 51, 55, and 60 (LC-FR1, LC-FR2, LC-FR3, and LC-FR4, respectively). In some embodiments, the anti-Siglec-8 antigen binding domain comprises a light chain variable domain framework sequence of SEQ ID NO:48, 51, 58, and 60 (LC-FR1, LC-FR2, LC-FR3, and LC-FR4, respectively).

[0106] In one embodiment, an anti-Siglec-8 antigen binding domain comprises a heavy chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the HC-FR1-HC-FR4 sequences SEQ ID NOs:26-29 (HC-FR1), SEQ ID NOs:31-36 (HC-FR2), SEQ ID NOs:38-43 (HC-FR3), and SEQ ID NOs:45 or 46 (HC-FR4), respectively; the HVR-H1 comprises the amino acid sequence of SEQ ID NO:61; the HVR-H2 comprises the amino acid sequence of SEQ ID NO:62; and the HVR-H3 comprises an amino acid sequence of SEQ ID NO:63. In one embodiment, an anti-Siglec-8 antigen binding domain comprises a heavy chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the HC-FR1-HC-FR4 sequences SEQ ID NOs:26-29 (HC-FR1), SEQ ID NOs:31-36 (HC-FR2), SEQ ID NOs:38-43 (HC-FR3), and SEQ ID NOs:45 or 46 (HC-FR4), respectively; the HVR-H1 comprises the amino acid sequence of SEQ ID NO:61; the HVR-H2 comprises the amino acid sequence of SEQ ID NO:62; and the HVR-H3 comprises an amino acid sequence selected from SEQ ID NOs:67-70. In one embodiment, an anti-Siglec-8 antigen binding domain comprises a light chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the LC-FR1-LC-FR4 sequences SEQ ID NOs:48 or 49 (LC-FR1), SEQ ID NOs:51-53 (LC-FR2), SEQ ID NOs:55-58 (LC-FR3), and SEQ ID NO:60 (LC-FR4), respectively; the HVR-L1 comprises the amino acid sequence of SEQ ID NO:64; the HVR-L2 comprises the amino acid sequence of SEQ ID NO:65; and the HVR-L3 comprises an

amino acid sequence of SEQ ID NO:66. In one embodiment, an anti-Siglec-8 antigen binding domain comprises a light chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the LC-FR1-LC-FR4 sequences SEQ ID NOs:48 or 49 (LC-FR1), SEQ ID NOs:51-53 (LC-FR2), SEQ ID NOs:55-58 (LC-FR3), and SEQ ID NO:60 (LC-FR4), respectively; the HVR-L1 comprises the amino acid sequence of SEQ ID NO:64; the HVR-L2 comprises the amino acid sequence of SEQ ID NO:65; and the HVR-L3 comprises an amino acid sequence of SEQ ID NO:71. In one embodiment of these anti-Siglec-8 antigen binding domains, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:2-10 and the light chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:16-22. In one embodiment of these anti-Siglec-8 antigen binding domains, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:2-10 and the light chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:23 or 24. In one embodiment of these anti-Siglec-8 antigen binding domains, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:11-14 and the light chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:16-22. In one embodiment of these anti-Siglec-8 antigen binding domains, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:11-14 and the light chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:23 or 24. In one embodiment of these anti-Siglec-8 antigen binding domains, the heavy chain variable domain comprises an amino acid sequence of SEQ ID NO:6 and the light chain variable domain comprises an amino acid sequence of SEQ ID NO:16. In one embodiment of these anti-Siglec-8 antigen binding domains, the heavy chain variable domain comprises an amino acid sequence of SEQ ID NO:6 and the light chain variable domain comprises an amino acid sequence of SEQ ID NO:21.

[0107] In some embodiments, the heavy chain HVR sequences comprise the following:

- a) HVR-H1 (IYGAH (SEQ ID NO:61));
- b) HVR-H2 (VIWAGGSTNYNSALMS (SEQ ID NO:62)); and
- c) HVR-H3 (DGSSPYYYSMEY (SEQ ID NO:63); DGSSPYYYGMEY (SEQ ID NO:67); DGSSPYYYSMDY (SEQ ID NO:68); DGSSPYYYSMEV (SEQ ID NO:69); or DGSSPYYYGMDV (SEQ ID NO:70)).

[0108] In some embodiments, the heavy chain HVR sequences comprise the following:

- a) HVR-H1 (SYAMS (SEQ ID NO:88); DYYMY (SEQ ID NO:89); or SSWMN (SEQ ID NO:90));
- b) HVR-H2 (IISSGGSYTYYSDSVKG (SEQ ID NO:91); RIAPEDGDTEYAPKFQG (SEQ ID NO:92); or QIYPGDDYTNYNGKFKG (SEQ ID NO:93)); and c) HVR-H3 (HETAQAAWFAY (SEQ ID NO:94); EGNYYGSSILDY (SEQ ID NO:95); or LGPYGPFAD (SEQ ID NO:96)).

[0109] In some embodiments, the heavy chain FR sequences comprise the following:

- a) HC-FR1 (EVQLVESGGGLVQPGGSLRLSCAASGFSLT (SEQ ID NO:26); EVQLVESGGGLVQPGGSLRLSCAVSGFSLT (SEQ ID NO:27); QVQLQESGPGLVKPSETLSLTCTVSGGSIS (SEQ ID NO:28); or QVQLQESGPGLVKPSETLSLTCTVSGFSLT (SEQ ID NO:29));
- b) HC-FR2 (WVRQAPGKGLEWVS (SEQ ID NO:31); WVRQAPGKGLEWLG (SEQ ID NO:32); WVRQAPGKGLEWLS (SEQ ID NO:33); WVRQAPGKGLEWVG (SEQ ID NO:34); WIRQPPGKGLEWIG (SEQ ID NO:35); or WVRQPPGKGLEWLG (SEQ ID NO:36));
- c) HC-FR3 (RFTISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:38); RLSISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:39); RLTIISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:40); RFSISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:41); RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO:42); or RLSISKDNSKNQVSLKLSSVTAADTAVYYCAR (SEQ ID NO:43)); and
- d) HC-FR4 (WGQGTTVTVSS (SEQ ID NO:45); or WGQGTLVTVSS (SEQ ID NO:46)).

[0110] In some embodiments, the light chain HVR sequences comprise the following:

- a) HVR-L1 (SATSSVSYMH (SEQ ID NO:64));
- b) HVR-L2 (STSNLAS (SEQ ID NO:65)); and
- c) HVR-L3 (QQRSSYPFT (SEQ ID NO:66); or QQRSSYPYT (SEQ ID NO:71)).

[0111] In some embodiments, the light chain HVR sequences comprise the following:

- a) HVR-L1 (SASSSVSYMH (SEQ ID NO:97); RASQDITNYLN (SEQ ID NO:98); or SASSSVSYMY (SEQ ID NO:99));
- b) HVR-L2 (DTSKLAY (SEQ ID NO:100); FTSRLHS (SEQ ID NO:101); or DTSSLAS (SEQ ID NO:102)); and

c) HVR-L3 (QQWSSNPPT (SEQ ID NO:103); QQGNTLPWT (SEQ ID NO:104); or QQWNSDPYT (SEQ ID NO:105)).

[0112] In some embodiments, the anti-Siglec-8 antigen binding domain comprises:

a heavy chain variable region comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:88, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:91, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:94; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:97, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:100, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:103;

a heavy chain variable region comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:89, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:92, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:95; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:98, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:101, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:104; or

a heavy chain variable region comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:90, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:93, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:96; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:99, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:102, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:105.

[0113] In some embodiments, the light chain FR sequences comprise the following:

a) LC-FR1 (EIVLTQSPATLSLSPGERATLSC (SEQ ID NO:48); or EIILTQSPATLSLSPGERATLSC (SEQ ID NO:49));

b) LC-FR2 (WFQQKPGQAPRLLIY (SEQ ID NO:51); WFQQKPGQAPRLWIY (SEQ ID NO:52); or WYQQKPGQAPRLLIY (SEQ ID NO: 53));

c) LC-FR3 (GIPARFSGSGSGTDFTLTSSLEPEDFAVYYC (SEQ ID NO:55); GVPARFSGSGSGTDYTLTSSLEPEDFAVYYC (SEQ ID NO:56); GVPARFSGSGSGTDFTLTSSLEPEDFAVYYC (SEQ ID NO:57); or GIPARFSGSGSGTDYTLTSSLEPEDFAVYYC (SEQ ID NO:58)); and

d) LC-FR4 (FGPGTKLDIK (SEQ ID NO:60)).

[0114] In some embodiments, provided herein is an anti-Siglec-8 antigen binding domain that binds to human Siglec-8, wherein the anti-Siglec-8 antigen binding domain comprises a heavy chain variable region and a light chain variable region, wherein the antibody comprises:

(a) heavy chain variable domain comprising:

- (1) an HC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:26-29;
- (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61;
- (3) an HC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:31-36;
- (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62;
- (5) an HC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:38-43;
- (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and
- (7) an HC-FR4 comprising the amino acid sequence selected from SEQ ID NOs:45-46, and/or

(b) a light chain variable domain comprising:

- (1) an LC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:48-49;
- (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64;
- (3) an LC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:51-53;
- (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65;
- (5) an LC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:55-58;
- (6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and
- (7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60.

[0115] In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain selected from SEQ ID NOs:2-10 and/or comprising a light chain variable domain selected from SEQ ID NOs:16-22. In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain selected from SEQ ID NOs:2-14 and/or comprising a light chain variable domain selected from SEQ ID NOs:16-24. In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain selected from SEQ ID NOs:2-10 and/or comprising a light chain variable domain selected from SEQ ID NO:23 or 24. In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain selected from SEQ ID NOs:11-14 and/or comprising a light chain variable domain selected from SEQ ID NOs:16-22. In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain selected from SEQ ID NOs:11-14 and/or comprising a light chain

variable domain selected from SEQ ID NO:23 or 24. In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain of SEQ ID NO:6 and/or comprising a light chain variable domain selected from SEQ ID NO:16 or 21. In some embodiments, the anti-Siglec-8 antigen binding domain is a human, humanized, or chimeric antigen binding domain.

[0116] In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain selected from SEQ ID NOs:106-108 and/or comprising a light chain variable domain selected from SEQ ID NOs:109-111. In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain of SEQ ID NO:106 and/or comprising a light chain variable domain of SEQ ID NO:109. In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain of SEQ ID NO:107 and/or comprising a light chain variable domain of SEQ ID NO:110. In one aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain of SEQ ID NO:108 and/or comprising a light chain variable domain of SEQ ID NO:111.

[0117] In some embodiments, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs:2-14. In some embodiments, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs:106-108. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, insertions, or deletions relative to the reference sequence, but an anti-Siglec-8 antigen binding domain comprising that amino acid sequence retains the ability to bind to human Siglec-8. In some embodiments, the substitutions, insertions, or deletions (e.g., 1, 2, 3, 4, or 5 amino acids) occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-Siglec-8 antigen binding domain comprises a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO:6. In some embodiments, an anti-Siglec-8 antigen binding domain comprises a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NOs:106-108.

[0118] In some embodiments, provided herein is an anti-Siglec-8 antigen binding domain comprising a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs:16-24. In some embodiments, provided herein is an anti-Siglec-8 antigen binding domain comprising a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs:109-111. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, insertions, or deletions relative to the reference sequence, but an anti-Siglec-8 antigen binding domain comprising that amino acid sequence retains the ability to bind to human Siglec-8. In some embodiments, the substitutions, insertions, or deletions (e.g., 1, 2, 3, 4, or 5 amino acids) occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-Siglec-8 antigen binding domain comprises a light chain variable domain comprising an amino acid sequence of SEQ ID NO:16 or 21. In some embodiments, an anti-Siglec-8 antigen binding domain comprises a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NOs:109-111.

[0119] In one aspect, the present disclosure provides an anti-Siglec-8 antigen binding domain comprising (a) one, two, or three VH HVRs selected from those shown in Table 1 and/or (b) one, two, or three VL HVRs selected from those shown in Table 1.

[0120] In one aspect, the present disclosure provides an anti-Siglec-8 antigen binding domain comprising (a) one, two, or three VH HVRs selected from those shown in Table 2 and/or (b) one, two, or three VL HVRs selected from those shown in Table 2.

[0121] In one aspect, the present disclosure provides an anti-Siglec-8 antigen binding domain comprising (a) one, two, three or four VH FRs selected from those shown in Table 3 and/or (b) one, two, three or four VL FRs selected from those shown in Table 3.

[0122] In some embodiments, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable domain and/or a light chain variable domain of an antibody shown in Table 4, for example, HAKA antibody, HAKB antibody, HAKC antibody, etc.

Table 1. Amino acid sequences of HVRs of antibodies

Antibody Chain	HVR1	HVR2	HVR3
<i>2E2 antibody</i>			

Heavy chain	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSM EY SEQ ID NO:63
Light chain	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPFT SEQ ID NO:66
<i>Humanized Heavy Chain Variants 2E2 RHA, 2E2 RHB, 2E2 RHC, 2E2 RHD, 2E2 RHE, 2E2 RHF, 2E2 RHG, 2E2 RHA2, and 2E2 RHB2</i>			
Heavy chain	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSM EY SEQ ID NO:63
<i>Humanized Light Chain Variants 2E2 RKA, 2E2 RKB, 2E2 RKC, 2E2 RKD, 2E2 RKE, 2E2 RKF, and 2E2 RKG</i>			
Light chain	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPFT SEQ ID NO:66
<i>Humanized Heavy Chain Variants 2E2 RHE S-G, 2E2 RHE E-D, 2E2 RHE Y-V, and 2E2 RHE triple</i>			
2E2 RHE S-G	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYGMEY SEQ ID NO:67
2E2 RHE E-D	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSM DY SEQ ID NO:68
2E2 RHE Y-V	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSM EV SEQ ID NO:69
2E2 RHE triple	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYGM DV SEQ ID NO:70
<i>Humanized Light Chain Variants 2E2 RKA F-Y and 2E2 RKF F-Y</i>			
2E2 RKA F-Y	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPYT SEQ ID NO:71
2E2 RKF F-Y	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPYT SEQ ID NO:71

Table 2. Amino acid sequences of HVRs from murine 1C3, 1H10, and 4F11 antibodies

Antibody	Chain	HVR1	HVR2	HVR3
1C3	Heavy Chain	SYAMS SEQ ID NO:88	IISGGSYTYYS DSVKG SEQ ID NO:91	HETAQA AWFAY SEQ ID NO:94
1H10	Heavy Chain	DYYMY SEQ ID NO:89	RIAPEDGDTEYAPKFQG SEQ ID NO:92	EGNYYGSSILDY SEQ ID NO:95
4F11	Heavy Chain	SSWMN SEQ ID NO:90	QIYPGDDYTNYNGKFKG SEQ ID NO:93	LGPYGP FAD SEQ ID NO:96
1C3	Light Chain	SASSSVSYMH SEQ ID NO:97	DTSKLAY SEQ ID NO:100	QQWSSNPPT SEQ ID NO:103
1H10	Light Chain	RASQDITNYLN	FTSRLHS	QQGNTLPWT

		SEQ ID NO:98	SEQ ID NO:101	SEQ ID NO:104
4F11	Light Chain	SASSSVSYMY SEQ ID NO:99	DTSSLAS SEQ ID NO:102	QQWNSDPYT SEQ ID NO:105

Table 3. Amino acid sequences of FRs of antibodies

Heavy Chain	FR1	FR2	FR3	FR4
2E2	QVQLKESGPGLVA PSQSL SITCTVSGFS LT (SEQ ID NO:25)	WVRQPPGKGLEW LG (SEQ ID NO:30)	RLSISKDNSKSQVF LKINSLQTDDTAL YYCAR (SEQ ID NO:37)	WGQGTSTVTVSS (SEQ ID NO:44)
2E2 RHA	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHB	EVQLVESGGGLVQ PGGSLRLSCAVSGF SLT (SEQ ID NO:27)	WVRQAPGKGLEW LG (SEQ ID NO:32)	RLSISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:39)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHC	EVQLVESGGGLVQ PGGSLRLSCAVSGF SLT (SEQ ID NO:27)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHD	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW LS (SEQ ID NO:33)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHE	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VG (SEQ ID NO:34)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHF	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RLTISKDNSKNTV YLQMNSLRAEDTA VYYCAR (SEQ ID NO:40)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHG	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RFSISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:41)	WGQGTTVTVSS (SEQ ID NO:45)

2E2 RHA2	QVQLQESGPGLVK PSETLSLTCTVSGG SIS (SEQ ID NO:28)	WIRQPPGKGLEWI G (SEQ ID NO:35)	RVTISVDTSKNQFS LKLSSVTAADTAV YYCAR (SEQ ID NO:42)	WGQGTLLTVVSS (SEQ ID NO:46)
2E2 RHB2	QVQLQESGPGLVK PSETLSLTCTVSGF SLT (SEQ ID NO:29)	WVRQPPGKGLEW LG (SEQ ID NO:36)	RLSISKDNSKNQVS LKLSSVTAADTAV YYCAR (SEQ ID NO:43)	WGQGTLLTVVSS (SEQ ID NO:46)
2E2 RHE S-G	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VG (SEQ ID NO:34)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTTVTVSS (SEQ ID NO:45)
2E2 RHE E-D	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VG (SEQ ID NO:34)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTTVTVSS (SEQ ID NO:45)
2E2 RHE Y-V	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VG (SEQ ID NO:34)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTTVTVSS (SEQ ID NO:45)
2E2 RHE triple	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VG (SEQ ID NO:34)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTTVTVSS (SEQ ID NO:45)
Light Chain	FR1	FR2	FR3	FR4
2E2	QIILTQSPAIMSASP GEKVSITC (SEQ ID NO:47)	WFQQKPGTSPKLW IY (SEQ ID NO:50)	GVPVRFSGSGSGTS YSLTISRMEAEDA ATYYC (SEQ ID NO:54)	FGSGTKLEIK (SEQ ID NO:59)
RKA	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRL IY (SEQ ID NO:51)	GIPARFSGSGSGTD FTLTISSELPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKB	EIILTQSPATLSLSP GERATLSC (SEQ ID NO:49)	WFQQKPGQAPRL WIY (SEQ ID NO:52)	GVPARFSGSGSGT DYTLTISSELPEDF AVYYC (SEQ ID NO:56)	FGPGTKLDIK (SEQ ID NO:60)

RKC	EILTQSPATLSLSP GERATLSC (SEQ ID NO:49)	WFQQKPGQAPRL IY (SEQ ID NO:51)	GIPARFSGSGSGTD FTLTISSLEPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKD	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRL WIY (SEQ ID NO:52)	GIPARFSGSGSGTD FTLTISSLEPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKE	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRL IY (SEQ ID NO:51)	GVPARFSGSGSGT DFTLTISSLEPEDFA VYYC (SEQ ID NO:57)	FGPGTKLDIK (SEQ ID NO:60)
RKF	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRL IY (SEQ ID NO:51)	GIPARFSGSGSGTD YTLTSSLEPEDFA VYYC (SEQ ID NO:58)	FGPGTKLDIK (SEQ ID NO:60)
RKG	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WYQQKPGQAPRL LIY (SEQ ID NO:53)	GIPARFSGSGSGTD FTLTISSLEPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKA F-Y	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRL IY (SEQ ID NO:51)	GIPARFSGSGSGTD FTLTISSLEPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKF F-Y	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRL IY (SEQ ID NO:51)	GIPARFSGSGSGTD YTLTSSLEPEDFA VYYC (SEQ ID NO:58)	FGPGTKLDIK (SEQ ID NO:60)

Table 4. Amino acid sequences of variable regions of antibodies

Antibody Name	Variable Heavy Chain	Variable Light Chain
ch2C4	ch2C4 VH	ch2C4 VK
ch2E2	ch2E2 VH (SEQ ID NO:1)	ch2E2 VK (SEQ ID NO:15)
cVHKA	ch2E2 VH (SEQ ID NO:1)	2E2 RKA (SEQ ID NO:16)

cVHKB	ch2E2 VH (SEQ ID NO:1)	2E2 RKB (SEQ ID NO:17)
HAcVK	2E2 RHA (SEQ ID NO:2)	ch2E2 VK (SEQ ID NO:15)
HBcVK	2E2 RHB (SEQ ID NO:3)	ch2E2 VK (SEQ ID NO:15)
HAKA	2E2 RHA (SEQ ID NO:2)	2E2 RKA (SEQ ID NO:16)
HAKB	2E2 RHA (SEQ ID NO:2)	2E2 RKB (SEQ ID NO:17)
HAKC	2E2 RHA (SEQ ID NO:2)	2E2 RKC (SEQ ID NO:18)
HAKD	2E2 RHA (SEQ ID NO:2)	2E2 RKD (SEQ ID NO:19)
HAKE	2E2 RHA (SEQ ID NO:2)	2E2 RKE (SEQ ID NO:20)
HAKF	2E2 RHA (SEQ ID NO:2)	2E2 RKF (SEQ ID NO:21)
HAKG	2E2 RHA (SEQ ID NO:2)	2E2 RKG (SEQ ID NO:22)
HBKA	2E2 RHB (SEQ ID NO:3)	2E2 RKA (SEQ ID NO:16)
HBKB	2E2 RHB (SEQ ID NO:3)	2E2 RKB (SEQ ID NO:17)
HBKC	2E2 RHB (SEQ ID NO:3)	2E2 RKC (SEQ ID NO:18)
HBKD	2E2 RHB (SEQ ID NO:3)	2E2 RKD (SEQ ID NO:19)
HBKE	2E2 RHB (SEQ ID NO:3)	2E2 RKE (SEQ ID NO:20)
HBKF	2E2 RHB (SEQ ID NO:3)	2E2 RKF (SEQ ID NO:21)
HBKG	2E2 RHB (SEQ ID NO:3)	2E2 RKG (SEQ ID NO:22)
HCKA	2E2 RHC (SEQ ID NO:4)	2E2 RKA (SEQ ID NO:16)
HCKB	2E2 RHC (SEQ ID NO:4)	2E2 RKB (SEQ ID NO:17)
HCKC	2E2 RHC (SEQ ID NO:4)	2E2 RKC (SEQ ID NO:18)
HCKD	2E2 RHC (SEQ ID NO:4)	2E2 RKD (SEQ ID NO:19)
HCKE	2E2 RHC (SEQ ID NO:4)	2E2 RKE (SEQ ID NO:20)
HCKF	2E2 RHC (SEQ ID NO:4)	2E2 RKF (SEQ ID NO:21)
HCKG	2E2 RHC (SEQ ID NO:4)	2E2 RKG (SEQ ID NO:22)
HDKA	2E2 RHD (SEQ ID NO:5)	2E2 RKA (SEQ ID NO:16)
HDKB	2E2 RHD (SEQ ID NO:5)	2E2 RKB (SEQ ID NO:17)
HDKC	2E2 RHD (SEQ ID NO:5)	2E2 RKC (SEQ ID NO:18)

HDKD	2E2 RHD (SEQ ID NO:5)	2E2 RKD (SEQ ID NO:19)
HDKE	2E2 RHD (SEQ ID NO:5)	2E2 RKE (SEQ ID NO:20)
HDKF	2E2 RHD (SEQ ID NO:5)	2E2 RKF (SEQ ID NO:21)
HDKG	2E2 RHD (SEQ ID NO:5)	2E2 RKG (SEQ ID NO:22)
HEKA	2E2 RHE (SEQ ID NO:6)	2E2 RKA (SEQ ID NO:16)
HEKB	2E2 RHE (SEQ ID NO:6)	2E2 RKB (SEQ ID NO:17)
HEKC	2E2 RHE (SEQ ID NO:6)	2E2 RKC (SEQ ID NO:18)
HEKD	2E2 RHE (SEQ ID NO:6)	2E2 RKD (SEQ ID NO:19)
HEKE	2E2 RHE (SEQ ID NO:6)	2E2 RKE (SEQ ID NO:20)
HEKF	2E2 RHE (SEQ ID NO:6)	2E2 RKF (SEQ ID NO:21)
HEKG	2E2 RHE (SEQ ID NO:6)	2E2 RKG (SEQ ID NO:22)
HFKA	2E2 RHF (SEQ ID NO:7)	2E2 RKA (SEQ ID NO:16)
HFKB	2E2 RHF (SEQ ID NO:7)	2E2 RKB (SEQ ID NO:17)
HFKC	2E2 RHF (SEQ ID NO:7)	2E2 RKC (SEQ ID NO:18)
HFKD	2E2 RHF (SEQ ID NO:7)	2E2 RKD (SEQ ID NO:19)
HFKE	2E2 RHF (SEQ ID NO:7)	2E2 RKE (SEQ ID NO:20)
HFKF	2E2 RHF (SEQ ID NO:7)	2E2 RKF (SEQ ID NO:21)
HFKG	2E2 RHF (SEQ ID NO:7)	2E2 RKG (SEQ ID NO:22)
HGKA	2E2 RHG (SEQ ID NO:8)	2E2 RKA (SEQ ID NO:16)
HGKB	2E2 RHG (SEQ ID NO:8)	2E2 RKB (SEQ ID NO:17)
HGKC	2E2 RHG (SEQ ID NO:8)	2E2 RKC (SEQ ID NO:18)
HGKD	2E2 RHG (SEQ ID NO:8)	2E2 RKD (SEQ ID NO:19)
HGKE	2E2 RHG (SEQ ID NO:8)	2E2 RKE (SEQ ID NO:20)
HGKF	2E2 RHG (SEQ ID NO:8)	2E2 RKF (SEQ ID NO:21)
HGHG	2E2 RHG (SEQ ID NO:8)	2E2 RKG (SEQ ID NO:22)
HA2KA	2E2 RHA2 (SEQ ID NO:9)	2E2 RKA (SEQ ID NO:16)
HA2KB	2E2 RHA2 (SEQ ID NO:9)	2E2 RKB (SEQ ID NO:17)

HB2KA	2E2 RHB2 (SEQ ID NO:10)	2E2 RKA (SEQ ID NO:16)
HB2KB	2E2 RHB2 (SEQ ID NO:10)	2E2 RKB (SEQ ID NO:17)
HA2KF	2E2 RHA2 (SEQ ID NO:9)	2E2 RKF (SEQ ID NO:21)
HB2KF	2E2 RHB2 (SEQ ID NO:10)	2E2 RKF (SEQ ID NO:21)
HA2KC	2E2 RHA2 (SEQ ID NO:9)	2E2 RKC (SEQ ID NO:18)
HA2KD	2E2 RHA2 (SEQ ID NO:9)	2E2 RKD (SEQ ID NO:19)
HA2KE	2E2 RHA2 (SEQ ID NO:9)	2E2 RKE (SEQ ID NO:20)
HA2KF	2E2 RHA2 (SEQ ID NO:9)	2E2 RKF (SEQ ID NO:21)
HA2KG	2E2 RHA2 (SEQ ID NO:9)	2E2 RKG (SEQ ID NO:22)
HB2KC	2E2 RHB2 (SEQ ID NO:10)	2E2 RKC (SEQ ID NO:18)
HB2KD	2E2 RHB2 (SEQ ID NO:10)	2E2 RKD (SEQ ID NO:19)
HB2KE	2E2 RHB2 (SEQ ID NO:10)	2E2 RKE (SEQ ID NO:20)
HA2KFmut	2E2 RHA2 (SEQ ID NO:9)	2E2 RKF F-Y mut (SEQ ID NO:24)
HB2KFmut	2E2 RHB2 (SEQ ID NO:10)	2E2 RKF F-Y mut (SEQ ID NO:24)
HEKAmut	2E2 RHE (SEQ ID NO:6)	2E2 RKA F-Y mut (SEQ ID NO:23)
HEKFmut	2E2 RHE (SEQ ID NO:6)	2E2 RKF F-Y mut (SEQ ID NO:24)
HAKFmut	2E2 RHA (SEQ ID NO:2)	2E2 RKF F-Y mut (SEQ ID NO:24)
HBKFmut	2E2 RHB (SEQ ID NO:3)	2E2 RKF F-Y mut (SEQ ID NO:24)
HCKFmut	2E2 RHC (SEQ ID NO:4)	2E2 RKF F-Y mut (SEQ ID NO:24)
HDKFmut	2E2 RHD (SEQ ID NO:5)	2E2 RKF F-Y mut (SEQ ID NO:24)
HFKFmut	2E2 RHF (SEQ ID NO:7)	2E2 RKF F-Y mut (SEQ ID NO:24)
HGKFmut	2E2 RHG (SEQ ID NO:8)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE Y-VKA	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKA (SEQ ID NO:16)
RHE Y-VKB	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKB (SEQ ID NO:17)
RHE Y-VKC	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKC (SEQ ID NO:18)
RHE Y-VKD	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKD (SEQ ID NO:19)
RHE Y-VKE	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKE (SEQ ID NO:20)

RHE Y-VKF	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKF (SEQ ID NO:21)
RHE Y-VKG	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKG (SEQ ID NO:22)
RHE E-DKA	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKA (SEQ ID NO:16)
RHE E-DKB	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKB (SEQ ID NO:17)
RHE E-DKC	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKC (SEQ ID NO:18)
RHE E-DKD	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKD (SEQ ID NO:19)
RHE E-DKE	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKE (SEQ ID NO:20)
RHE E-DKF	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKF (SEQ ID NO:21)
RHE E-DKG	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKG (SEQ ID NO:22)
RHE E-DKFmut	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE S-GKA	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKA (SEQ ID NO:16)
RHE S-GKB	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKB (SEQ ID NO:17)
RHE S-GKC	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKC (SEQ ID NO:18)
RHE S-GKD	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKD (SEQ ID NO:19)
RHE S-GKE	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKE (SEQ ID NO:20)
RHE S-GKF	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKF (SEQ ID NO:21)
RHE S-GKG	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKG (SEQ ID NO:22)
RHE Triple-KA	2E2 RHE triple (SEQ ID NO:14)	2E2 RKA (SEQ ID NO:16)
RHE Triple-KB	2E2 RHE triple (SEQ ID NO:14)	2E2 RKB (SEQ ID NO:17)
RHE Triple-KC	2E2 RHE triple (SEQ ID NO:14)	2E2 RKC (SEQ ID NO:18)
RHE Triple-KD	2E2 RHE triple (SEQ ID NO:14)	2E2 RKD (SEQ ID NO:19)
RHE Triple-KE	2E2 RHE triple (SEQ ID NO:14)	2E2 RKE (SEQ ID NO:20)
RHE Triple-KF	2E2 RHE triple (SEQ ID NO:14)	2E2 RKF (SEQ ID NO:21)
RHE Triple-KG	2E2 RHE triple (SEQ ID NO:14)	2E2 RKG (SEQ ID NO:22)
RHE Triple-KFmut	2E2 RHE triple (SEQ ID NO:14)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE Y-VKFmut	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE E-DKFmut	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKF F-Y mut (SEQ ID NO:24)

[0123] Many definitions for CDR or HVR sequences of an antibody variable domain are known in the art and may be used to describe an antibody of the present disclosure, *e.g.*, by CDR/HVR sequences. In some embodiments, antibody CDR/HVR sequences are defined as in Kabat (*see, e.g., Kabat et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institute of Health, Bethesda, MD (1991)). In some embodiments, antibody CDR/HVR sequences are defined as in Chothia (*see, e.g., Chothia and Lesk J. Mol. Biol.* 196:901-917 (1987)). In some embodiments, antibody CDR/HVR sequences are defined as in IMGT (*see, e.g., Lefranc, M.P. (1999) The Immunologist* 7:132-136). In some embodiments, CDR/HVR sequences of a single antibody are defined as by mixing two or more definitions, *e.g.*, Kabat, Chothia, and/or IMGT.

[0124] In another aspect, provided herein is an anti-Siglec-8 antigen binding domain comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of GFSLTIIY (SEQ ID NO:296), (ii) HVR-H2 comprising the amino acid sequence of WAGGS (SEQ ID NO:297), and (iii) HVR-H3 comprising the amino acid sequence of DGSSPYYSMEY (SEQ ID NO:63); and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SATSSVSYMH (SEQ ID NO:64), (ii) HVR-L2 comprising the amino acid sequence of STSNLAS (SEQ ID NO:65), and (iii) HVR-L3 comprising the amino acid sequence of QQRSSYPFT (SEQ ID NO:66). In some embodiments, the antibody HVR sequences are defined as in Chothia.

[0125] In some aspects, an anti-Siglec-8 antigen binding domain or bispecific antibody described herein binds to human Siglec-8 with about the same or higher affinity and/or higher avidity as compared to mouse antibody 2E2 and/or mouse antibody 2C4. In certain embodiments, an anti-Siglec-8 antigen binding domain or bispecific antibody provided herein has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 150\text{ nM}$, $\leq 100\text{ nM}$, $\leq 50\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (*e.g.* 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M , *e.g.*, from 10^{-9} M to 10^{-13} M). In some embodiments, an anti-Siglec-8 antigen binding domain or bispecific antibody described herein binds to human Siglec-8 at about 1.5-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold or about 10-fold higher affinity than mouse antibody 2E2 and/or mouse antibody 2C4. In some embodiments, the anti-Siglec-8 antigen binding domain or bispecific antibody comprises a

heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16 or 21.

[0126] In one embodiment, the binding affinity of the anti-Siglec-8 antigen binding domain or bispecific antibody can be determined by a surface plasmon resonance assay. For example, the K_d or K_d value can be measured by using a BIAcore™-2000 or a BIAcore™-3000 (BIAcore, Inc., Piscataway, N.J.) at 25° C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore® Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Capture antibodies (e.g., anti-human-Fc) are diluted with 10 mM sodium acetate, pH 4.8, before injection at a flow rate of 30 µl/minute and further immobilized with an anti-Siglec-8 antibody. For kinetics measurements, two-fold serial dilutions of dimeric Siglec-8 are injected in PBS with 0.05% Tween 20 (PBST) at 25° C at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_d) is calculated as the ratio k_{off}/k_{on} . See, e.g., Chen, Y., et al., (1999) J. Mol. Biol. 293:865-881.

[0127] In another embodiment, biolayer interferometry may be used to determine the affinity of anti-Siglec-8 antigen binding domains or bispecific antibodies against Siglec-8. In an exemplary assay, Siglec-8-Fc tagged protein is immobilized onto anti-human capture sensors, and incubated with increasing concentrations of mouse, chimeric, or humanized anti-Siglec-8 Fab fragments to obtain affinity measurements using an instrument such as, for example, the Octet Red 384 System (ForteBio).

[0128] The binding affinity of the anti-Siglec-8 antigen binding domain or bispecific antibody can, for example, also be determined by the Scatchard analysis described in Munson et al., Anal. Biochem., 107:220 (1980) using standard techniques well known in the relevant art. See also Scatchard, G., Ann. N.Y. Acad. Sci. 51:660 (1947).

[0129] In some embodiments, the binding avidity of the anti-Siglec-8 antigen binding domain or bispecific antibody can be determined by a surface plasmon resonance assay. For example, the K_d or K_d value can be measured by using a BIAcore T100. Capture antibodies (e.g., goat-anti-human-Fc and goat-anti-mouse-Fc) are immobilized on a CM5 chip. Flow-cells can be immobilized with anti-human or with anti-mouse antibodies. The assay is conducted at a certain

temperature and flow rate, for example, at 25°C at a flow rate of 30 µl/min. Dimeric Siglec-8 is diluted in assay buffer at various concentrations, for example, at a concentration ranging from 15 nM to 1.88 pM. Antibodies are captured and high performance injections are conducted, followed by dissociations. Flow cells are regenerated with a buffer, for example, 50 mM glycine pH 1.5. Results are blanked with an empty reference cell and multiple assay buffer injections, and analyzed with 1:1 global fit parameters.

[0130] Competition-assays can be used to determine whether two antibodies bind the same epitope by recognizing identical or sterically overlapping epitopes or one antibody competitively inhibits binding of another antibody to the antigen. These assays are known in the art. Typically, antigen or antigen expressing cells is immobilized on a multi-well plate and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured. Common labels for such competition assays are radioactive labels or enzyme labels. In some embodiments, an anti-Siglec-8 antibody described herein competes with a 2E2 antibody described herein, for binding to the epitope present on the cell surface of a cell (*e.g.*, a mast cell). In some embodiments, an anti-Siglec-8 antigen binding domain or bispecific antibody described herein competes with an antibody comprising a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:1, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:15, for binding to the epitope present on the cell surface of a cell (*e.g.*, a mast cell). In some embodiments, an anti-Siglec-8 antigen binding domain or bispecific antibody described herein competes with a 2C4 antibody described herein, for binding to the epitope present on the cell surface of a cell (*e.g.*, a mast cell). In some embodiments, an anti-Siglec-8 antigen binding domain or bispecific antibody described herein competes with an antibody comprising a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:2 (as found in U.S. Pat. No. 8,207,305), and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4 (as found in U.S. Pat. No. 8,207,305), for binding to the epitope present on the cell surface of a cell (*e.g.*, a mast cell).

[0131] In some aspects, an anti-Siglec-8 antigen binding domain or bispecific antibody described herein has a melting temperature (T_m) of at least about 70°C, at least about 71°C, or at least about 72°C in a thermal shift assay. In an exemplary thermal shift assay, samples comprising a humanized anti-Siglec-8 antibody are incubated with a fluorescent dye (Sypro Orange) for 71 cycles with 1°C increase per cycle in a qPCR thermal cycler to determine the T_m . In some embodiments, the anti-Siglec-8 antigen binding domain or bispecific antibody has a

similar or higher T_m as compared to mouse 2E2 antibody and/or mouse 2C4 antibody. In some embodiments, the anti-Siglec-8 antigen binding domain or bispecific antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16 or 21. In some embodiments, the anti-Siglec-8 antigen binding domain or bispecific antibody has the same or higher T_m as compared to a chimeric 2C4 antibody. In some embodiments, the anti-Siglec-8 antigen binding domain or bispecific antibody has the same or higher T_m as compared to an antibody having a heavy chain comprising the amino acid sequence of SEQ ID NO:84 and a light chain comprising the amino acid sequence of SEQ ID NO:85.

[0132] In some embodiments, a bispecific antibody described herein depletes eosinophils and inhibits mast cells. Assays for assessing apoptosis of cells are well known in the art, for example staining with Annexin V and the TUNNEL assay.

[0133] In some embodiments, a bispecific antibody described herein induces ADCC activity. In some embodiments, a bispecific antibody described herein kills eosinophils expressing Siglec-8 by ADCC activity. In some embodiments, a composition comprises non-fucosylated (*i.e.*, afucosylated) bispecific antibodies. In some embodiments, a composition comprising non-fucosylated bispecific antibodies described herein enhances ADCC activity against Siglec-8 expressing eosinophils as compared to a composition comprising partially fucosylated anti-Siglec-8 antibodies. Assays for assessing ADCC activity are well known in the art and described herein. In an exemplary assay, to measure ADCC activity, effector cells and target cells are used. Examples of effector cells include natural killer (NK) cells, large granular lymphocytes (LGL), lymphokine-activated killer (LAK) cells and PBMC comprising NK and LGL, or leukocytes having Fc receptors on the cell surfaces, such as neutrophils, eosinophils and macrophages. Effector cells can be isolated from any source including individuals with a disease of interest (*e.g.*, mast cell gastritis, mast cell esophagitis, mast cell colitis, mast cell enteritis, mast cell duodenitis, and/or mast cell gastroenteritis). The target cell is any cell which expresses on the cell surface antigens that antibodies to be evaluated can recognize. An example of such a target cell is an eosinophil which expresses Siglec-8 on the cell surface. Another example of such a target cell is a cell line (*e.g.*, Ramos cell line) which expresses Siglec-8 on the cell surface (*e.g.*, Ramos 2C10)). Target cells can be labeled with a reagent that enables detection of cytotoxicity. Examples of reagents for labeling include a radio-active substance such as sodium chromate

(Na₂ ⁵¹CrO₄). See, e.g., *Immunology*, 14, 181 (1968); *J. Immunol. Methods.*, 172, 227 (1994); and *J. Immunol. Methods.*, 184, 29 (1995).

[0134] In an exemplary assay to assess ADCC and apoptotic activity of bispecific antibodies on mast cells, human mast cells are isolated from human tissues or biological fluids according to published protocols (Guhl et al., *Biosci. Biotechnol. Biochem.*, 2011, 75:382-384; Kulka et al., *In Current Protocols in Immunology*, 2001, (John Wiley & Sons, Inc.)) or differentiated from human hematopoietic stem cells, for example as described by Yokoi et al., *J Allergy Clin Immunol.*, 2008, 121:499-505. Purified mast cells are resuspended in Complete RPMI medium in a sterile 96-well U-bottom plate and incubated in the presence or absence of anti-Siglec-8 antibodies for 30 minutes at concentrations ranging between 0.0001 ng/ml and 10 µg/ml. Samples are incubated for a further 4 to 48 hours with and without purified natural killer (NK) cells or fresh PBL to induce ADCC. Cell-killing by apoptosis or ADCC is analyzed by flow cytometry using fluorescent conjugated antibodies to detect mast cells (CD117 and FcεR1) and Annexin-V and 7AAD to discriminate live and dead or dying cells. Annexin-V and 7AAD staining are performed according to manufacturer's instructions.

[00100] In some aspects, a bispecific antibody described herein inhibits mast cell-mediated activities. Mast cell tryptase has been used as a biomarker for total mast cell number and activation. For example, total and active tryptase as well as histamine, N-methyl histamine, and 11-beta-prostaglandin F2 can be measured in blood or urine to assess the reduction in mast cells. See, e.g., U.S. Patent Application Publication No. US 20110293631 for an exemplary mast cell activity assay.

Anti-Siglec-6 antigen binding domains

[0135] Certain aspects of the present disclosure relate to antigen binding domains that bind to human Siglec-6, *i.e.*, anti-Siglec-6 antigen binding domains. Antigen binding domains from any of the antibodies that bind to human Siglec-6 described in International Publication No. WO2023044390 may find use in a bispecific antibody of the present disclosure.

[0136] In some embodiments, the anti-Siglec-6 antigen binding domain is a humanized antigen binding domain that binds to Domain 1 of an extracellular domain of human Siglec-6, *e.g.*, comprising the amino acid sequence

QERRFQLEGPESLTVQEGLCVLVPCRLPTTLPASYYGYGYWFLEGADVVPVATNDPDEE

VQEETRGRFHLLWDP RRKNC SL SIRDARRRDNAA YFFRLKSKWMKYGYTSSKLSVRV
MAL THR (SEQ ID NO:121).

[0137] In some embodiments, the anti-Siglec-6 antigen binding domain binds to Domain 2 of an extracellular domain of human Siglec-6, *e.g.*, comprising the amino acid sequence
PNISIPGTLES GHPSNL TCSVPWVCEQGTPIFSWMSAAPTSLGPRTTQSSVL TITPRPQDH
STNLTCQVTFPGAGVTMERTIQLNVS YA (SEQ ID NO:122). In some embodiments, the antigen binding domain is a humanized or human antigen binding domain.

[0138] In some embodiments, the anti-Siglec-6 antigen binding domain binds to Domain 3 of an extracellular domain of human Siglec-6, *e.g.*, comprising the amino acid sequence
PQKVAISIFQGN SAAFKILQNTSSLPVLEGQALRLLCDADGNPPAHL SWFQGFALNATP
ISNTGVLELPQVGSAEEGDFTCRAQHPLGSLQISLSLFVHWKPEGRAGGV (SEQ ID
NO:123). In some embodiments, the antigen binding domain is a humanized or human antigen binding domain.

[0139] In some embodiments, the anti-Siglec-6 antigen binding domain comprises 1, 2, 3, 4, 5, or all 6 HVR sequences of a single anti-Siglec-6 antigen binding domain as set forth in Table 6. In some embodiments, the anti-Siglec-6 antigen binding domain comprises a VH region comprising 1, 2, or all 3 HVR sequences of a VH region of a single anti-Siglec-6 antigen binding domain as set forth in Table 6. In some embodiments, the anti-Siglec-6 antigen binding domain comprises a VL region comprising 1, 2, or all 3 HVR sequences of a VL region of a single anti-Siglec-6 antigen binding domain as set forth in Table 6. In some embodiments, the anti-Siglec-6 antigen binding domain is a human, humanized, or chimeric antigen binding domain.

Table 6. Anti-Siglec-6 antigen binding domain HVR sequences.

SEQ ID NO	Antibody Name	Sequence Description	Sequence
124	AK04	HVR-H1	GFSLT SYGVS
125		HVR-H2	VIWHDG STSYHSTLIS
126		HVR-H3	DGYSGTFAY
127		HVR-L1	TASSSVSSSYLH
128		HVR-L2	STSILAS
129		HVR-L3	HQYHRSPYT
130	AK05	HVR-H1	GYIFTSYWMY
131		HVR-H2	NVNPYSGDTNYNEKF KT
132		HVR-H3	GDGLYYYAMDY
133		HVR-L1	KASQDINSYLT
134		HVR-L2	RASRLVD
135		HVR-L3	LQYDEFPLT

136	AK02	HVR-H1	GYTFTNYIIH
137		HVR-H2	MIHPISGSTNYNEKLKI
138		HVR-H3	RYYGSSYWFYFDV
139		HVR-L1	RASSSVSYMN
140		HVR-L2	ATSNLAS
141		HVR-L3	QQWSSHPPT
142	AK14	HVR-H1	GYTFTTYGMS
143		HVR-H2	WINTYSGVPTYADDFKG
144		HVR-H3	LAYFGYYFDF
145		HVR-L1	KASQNVGSAVA
146		HVR-L2	SASNRYT
147		HVR-L3	QQYSSYPYT
148	AK11	HVR-H1	GFSFSTYVMS
149		HVR-H2	SISGGGSNTYYPDSVKG
150		HVR-H3	REVVVYWFYFDV
151		HVR-L1	RASENIYSNLA
152		HVR-L2	SAKNLAD
153		HVR-L3	QHFWTGPLT
154	AK15	HVR-H1	GFSLTSYGVS
155		HVR-H2	IIWGDGSTNYHSALIS
156		HVR-H3	GLSDWYFDV
157		HVR-L1	RASSSVSYMH
158		HVR-L2	ATSNLAS
159		HVR-L3	QQWSSNPYT
160	AK13	HVR-H1	GYTFTTYGMS
161		HVR-H2	WINTYSGVPTYADDFKG
162		HVR-H3	LAYFGYYFDF
163		HVR-L1	KASQNVGSAVT
164		HVR-L2	SASNRYT
165		HVR-L3	QQYSSYPYT
166	AK12	HVR-H1	GFSLTSYGVH
167		HVR-H2	VIWGGGSTDYNAGFIS
168		HVR-H3	ALSDWYFDV
169		HVR-L1	RASSSVSYMH
170		HVR-L2	ATSNLAS
171		HVR-L3	QQWSSNPLT
172	AK10	HVR-H1	GYTFTDYIMN
173		HVR-H2	DINPKIGDTIYNQKFKG
174		HVR-H3	WDYYGSSLFDY
175		HVR-L1	KASQDVTTAVA
176		HVR-L2	WASTRHT
177		HVR-L3	QQHYSTPYT
178	AK09	HVR-H1	GYTLTTYGMS
179		HVR-H2	WINTYSGVPTYADEFKG
180		HVR-H3	LGYYGYFYFDY
181		HVR-L1	RASQSIRNNLH
182		HVR-L2	YIFQIS
183		HVR-L3	QQSDSWPHVT
184	AK08	HVR-H1	GFSLTSYGVN
185		HVR-H2	VIWGDGSTNYHSALIS
186		HVR-H3	ALSDWYFDV

187	AK07	HVR-L1	RASSSVSYMH
188		HVR-L2	ATSNLAS
189		HVR-L3	QQWSSNPYT
190		HVR-H1	GYTFTDYKMH
191		HVR-H2	NINPNNGGTSYNQKFKG
192		HVR-H3	RYYGSSSSYFDY
193		HVR-L1	SVSSSVSYMH
194	AK06	HVR-L2	DTSKLAS
195		HVR-L3	QQWSSNPPT
196		HVR-H1	GFTFSDYGIH
197		HVR-H2	YITSVSSTIYYADTVKG
198		HVR-H3	LDWYFDV
199		HVR-L1	SANSSVSFMH
200		HVR-L2	DTSKLAS
201	AK03	HVR-L3	QQWSSNPLT
202		HVR-H1	GYTFTSYWMH
203		HVR-H2	TIYPGNSDSTYNQKFKG
204		HVR-H3	EGTVVANYYYGLDY
205		HVR-L1	RASSSVSYIH
206		HVR-L2	ATSNLAS
207		HVR-L3	QQWSSKPPT
208	AK01	HVR-H1	GFSLTYSVH
209		HVR-H2	VIWSGGSTDYNAVFIS
210		HVR-H3	YSYITMDY
211		HVR-L1	RSSQSLVLDNGNTYLH
212		HVR-L2	KVSNRFS
213		HVR-L3	SQSTHVPFT
214	AK16	HVR-H1	GFNIKDDYMH
215		HVR-H2	WIDLENGDTEYASKFQG
216		HVR-H3	PIGTVVAEGFAY
217		HVR-L1	RASENIYSNLA
218		HVR-L2	AATNLAD
219		HVR-L3	QHFWGTPYT
220	AK17	HVR-H1	GYTFTDYKMH
221		HVR-H2	NINPNNGGTSYNQKFKG
222		HVR-H3	RYYGSSSSYFDY
223		HVR-L1	RASQSIGTSIH
224		HVR-L2	YASESIS
225		HVR-L3	QQSNSWPTT
226	AK18	HVR-H1	GFTFSDYGMH
227		HVR-H2	YISSDSSTIYYADTVKG
228		HVR-H3	TIYYGNFYFDY
229		HVR-L1	SASSSVSSSLY
230		HVR-L2	SMSNLAS
231		HVR-L3	QQWSSNPLT

[0140] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:208, an HVR-H2

comprising the amino acid sequence of SEQ ID NO:209, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:210; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:211, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:212, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:213.

[0141] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:124, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:125, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:126; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:127, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:128, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:129.

[0142] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:202, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:203, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:204; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:205, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:206, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:207.

[0143] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:130, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:131, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:132; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:133, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:134, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:135.

[0144] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:196, an HVR-H2

comprising the amino acid sequence of SEQ ID NO:197, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:198; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:199, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:200, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:201.

[0145] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:136, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:137, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:138; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:139, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:140, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:141.

[0146] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:190, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:191, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:192; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:193, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:194, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:195.

[0147] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:172, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:173, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:174; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:175, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:176, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:177.

[0148] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:148, an HVR-H2

comprising the amino acid sequence of SEQ ID NO:149, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:150; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:151, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:152, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:153.

[0149] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:142, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:143, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:144; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:145, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:146, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:147.

[0150] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:154, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:155, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:156; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:157, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:158, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:159.

[0151] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:160, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:161, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:162; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:163, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:164, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:165.

[0152] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:166, an HVR-H2

comprising the amino acid sequence of SEQ ID NO:167, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:168; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:169, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:170, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:171.

[0153] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:178, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:179, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:180; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:181, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:182, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:183.

[0154] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:184, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:185, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:186; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:187, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:188, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:189.

[0155] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:214, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:215, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:216; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:217, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:218, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:219.

[0156] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:220, an HVR-H2

comprising the amino acid sequence of SEQ ID NO:221, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:222; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:223, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:224, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:225.

[0157] In some embodiments, the anti-Siglec-6 antigen binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:226, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:227, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:228; and wherein the VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:229, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:230, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:231.

[0158] In some embodiments, an anti-Siglec-6 antigen binding domain provided herein competes for binding to human Siglec-6 (*e.g.*, an ECD or sub-domain thereof of a human Siglec-6 protein) with a reference antibody, *e.g.*, an anti-Siglec-6 antibody of the present disclosure. In some embodiments, an anti-Siglec-6 antigen binding domain provided herein competes for binding to human Siglec-6 (*e.g.*, an ECD or sub-domain thereof of a human Siglec-6 protein) with one or more of the following anti-Siglec-6 antibodies described herein: AK04, AK05, AK02, AK14, AK11, AK15, AK13, AK12, AK10, AK09, AK08, AK07, AK06, AK03, AK01, AK16, AK17, and AK18. In some embodiments, an anti-Siglec-6 antigen binding domain provided herein competes for binding to Domain 1 of human Siglec-6 with one or more of the following anti-Siglec-6 antibodies described herein: AK04, AK05, AK02, AK07, AK06, AK03, and AK01. In some embodiments, an anti-Siglec-6 antigen binding domain provided herein competes for binding to Domain 2 of human Siglec-6 with one or more of the following anti-Siglec-6 antibodies described herein: AK10 and AK11. In some embodiments, an anti-Siglec-6 antigen binding domain provided herein competes for binding to Domain 3 of human Siglec-6 with one or more of the following anti-Siglec-6 antibodies described herein: AK09, AK08, AK12, AK13, AK14, and AK15.

[0159] In some embodiments, an anti-Siglec-6 antigen binding domain described herein binds to an extracellular domain (ECD) of a human Siglec-6 protein. In some embodiments, the Siglec-6 ECD comprises the amino acid sequence

QERRFQLEGPESLTVQEGLCVLVPCRLPTTLPASYYGYGYWFLEGADVPVATNDPDEE
VQEETRGRFHLLWDPRRKNC SLSIRDARRRDNAAYFFRLKSKWMKYGYTSSKLSVRV
MALTHRPNISIPGTLES GHPSNL TCSVPWVCEQGTPIFSWMSAAPTSLGPRTTQSSVLTIT
TPRPQDHSTNLTCQVTFPGAGVTMERTIQLNVSYAPQKVAISIFQGNSAAFKILQNTSSLP
VLEGQALRLLCDADGNPPAHL SWFQGF PALNATPISNTGVLELPQVGSAEEGDFTCRAQ
HPLGSLQISLSL FVHWKPEGRAGGV (SEQ ID NO:120).

[0160] In some embodiments, an anti-Siglec-6 antigen binding domain described herein binds to Domain 1, Domain 2, or Domain 3 of a human Siglec-6 protein (*e.g.*, an ECD of a human Siglec-6 protein). In some embodiments, an anti-Siglec-6 antigen binding domain described herein binds to Domain 1 of a human Siglec-6 protein (*e.g.*, an ECD of a human Siglec-6 protein). In some embodiments, Domain 1 comprises the amino acid sequence

QERRFQLEGPESLTVQEGLCVLVPCRLPTTLPASYYGYGYWFLEGADVPVATNDPDEE
VQEETRGRFHLLWDPRRKNC SLSIRDARRRDNAAYFFRLKSKWMKYGYTSSKLSVRV
MALTHR (SEQ ID NO:121). In some embodiments, an anti-Siglec-6 antigen binding domain

described herein binds to Domain 2 of a human Siglec-6 protein (*e.g.*, an ECD of a human Siglec-6 protein). In some embodiments, Domain 2 comprises the amino acid sequence
PNISIPGTLES GHPSNL TCSVPWVCEQGTPIFSWMSAAPTSLGPRTTQSSVLTITPRPQDH
STNLTCQVTFPGAGVTMERTIQLNVSYA (SEQ ID NO:122). In some embodiments, an
anti-Siglec-6 antigen binding domain described herein binds to Domain 3 of a human Siglec-6
protein (*e.g.*, an ECD of a human Siglec-6 protein). In some embodiments, Domain 3 comprises
the amino acid sequence

PQKVAISIFQGNSAAFKILQNTSSLPVLEGQALRLLCDADGNPPAHL SWFQGF PALNATP
ISNTGVLELPQVGSAEEGDFTCRAQHPLGSLQISLSL FVHWKPEGRAGGV (SEQ ID
NO:123).

[0161] In some embodiments, an anti-Siglec-6 antigen binding domain provided herein binds the same epitope on human Siglec-6 (*e.g.*, an ECD or sub-domain thereof of a human Siglec-6 protein) as an anti-Siglec-6 antibody of the present disclosure. In some embodiments, an anti-Siglec-6 antigen binding domain provided herein binds the same epitope on human Siglec-6 Domain 1, 2, or 3 as an anti-Siglec-6 antibody of the present disclosure.

[0162] Many definitions for CDR or HVR sequences of an antibody variable domain are known in the art and may be used to describe an antibody of the present disclosure, *e.g.*, by CDR/HVR sequences. In some embodiments, antibody CDR/HVR sequences are defined as in

Kabat (*see, e.g., Kabat et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institute of Health, Bethesda, MD (1991)). In some embodiments, antibody CDR/HVR sequences are defined as in Chothia (*see, e.g., Chothia and Lesk J. Mol. Biol.* 196:901-917 (1987)). In some embodiments, antibody CDR/HVR sequences are defined as in IMGT (*see, e.g., Lefranc, M.P. (1999) The Immunologist* 7:132-136). In some embodiments, CDR/HVR sequences of a single antibody are defined as by mixing two or more definitions, *e.g., Kabat, Chothia, and/or IMGT.*

[0163] In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:232 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:233. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK15 as described herein (*see, e.g., Table 7*) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK15 as described herein (*see, e.g., Table 7*). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:234 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:235. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK14 as described herein (*see, e.g., Table 7*) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK14 as described herein (*see, e.g., Table 7*). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:236 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:237. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK13 as described herein (*see, e.g., Table 7*) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK13 as described herein (*see, e.g., Table 7*). In some embodiments, an anti-Siglec-6 antigen binding domain of the present

disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:238 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:239. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK12 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK12 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:240 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:241. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK11 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK11 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:242 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:243. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK10 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK10 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:244 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:245. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK09 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK09 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present

disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:246 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:247. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK08 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK08 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:248 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:249. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK07 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK07 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:250 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:251. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK06 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK06 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:252 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:253. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK05 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK05 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present

disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:254 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:255. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK04 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK04 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:256 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:257. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK03 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK03 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:258 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:259. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK02 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK02 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:260 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:261. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK01 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK01 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present

disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:262 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:263. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK16 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK16 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:264 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:265. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK17 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK17 as described herein (*see, e.g.*, Table 7). In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:266 and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the amino acid sequence of SEQ ID NO:267. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VH domain sequence of AK18 as described herein (*see, e.g.*, Table 7) and/or a VL domain comprising 1, 2, or all 3 CDR or HVR sequences present in the VL domain sequence of AK18 as described herein (*see, e.g.*, Table 7). In some embodiments according to any of the embodiments described herein, the antibody is a humanized antibody.

[0164] In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:232 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:233. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:234 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:235. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the

amino acid sequence of SEQ ID NO:236 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:237. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:238 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:239. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:240 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:241. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:242 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:243. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:244 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:245. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:246 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:247. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:248 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:249. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:250 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:251. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:252 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:253. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:254 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:255. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:256 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:257. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:258 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:259. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the

amino acid sequence of SEQ ID NO:260 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:261. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:262 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:263. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:264 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:265. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain comprising the amino acid sequence of SEQ ID NO:266 and/or a VL domain comprising the amino acid sequence of SEQ ID NO:267. In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure comprises a VH domain and/or a VL domain of a single antigen binding domain as described in Table 7. In some embodiments according to any of the embodiments described herein, the antibody is a humanized antibody.

Table 7. Anti-Siglec-6 variable domain sequences.

SEQ ID NO	Antibody Name	Sequence Description	Sequence
232	AK15	VH	QVQLKESGPGLVAPSQSL SITCTVSGFSLT SYGVSWVRQPPGKGLEWLGIIWGDGSTN YHSALISRLSISKDKSKSQVFLKLNSLQAD DTATYYCAKGLSDWYFDVWGTGTTVTV SS
233		VL	QIVLSQSPAILSASPGEKVTMTCRASSVS YMHWYQQKPGSSPKWIYATSNLASGVP ARFSGSGSGTSYSLTISRVEAEDAATYYC QQWSSNPYTFGGGTKLEIK
234	AK14	VH	QIQLVQSGPELKKPGETVKISCKASGYTFT TYGMSWVKQAPGKGLKWMGWINTYSG VPTYADDFKGRFAFSLETSATTAYLQINN LKNEDTATYFCARLAYFGYYDFWQGQT TLTVSS
235		VL	DIVMTQSQKFMSTTVGDRVSFTCKASQN VGSAAWYQQKPGQSPKLLIYSASNRYT GVPDRFTGSGSGTDFTLTISNMQSEDLAD YFCQQYSSYPYTFGGGTKLEIK
236	AK13	VH	QIQLVQSGPELKKPGETVKISCKASGYTFT TYGMSWVKQAPGKGLKWMGWINTYSG VPTYADDFKGRFAFSLETSATTAYLQINN LKNEDTATYFCARLAYFGYYDFWQGQT TLTVSS
237		VL	DIVMTQSQKFMSTTVGDRVSFTCKASQN VGSVWYQQKPGQSPKLLIYSASNRYT GVPDRFTGSGSGTDFTLTISNMQSEDLAD YFCQQYSSYPYTFGGGTKLEIK

238	AK12	VH	QVQLKQSGPGLVQPSQSL SITCTVSGFSLT SYGVHWVRQSPGKGLEWLGVIWGGGST DYNAGFISRLSISKDNSKSQVFFKMNSLQ ADDTAIYYCARALSDWYFDVWGTGTTV TVSS
239		VL	QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWYQQKPGSSPKPWIYATSNLASGVP ARFSGSGSGTSYSLTISRVEAEDAATYYC QQWSSNPLTFGAGTKLELK
240	AK11	VH	EVMLVESGGGLVKPGSLKLSCAASGFSF STYVMSWVRQTPEKRLEWVASISGGGSN TYYPDSVKGRFTISRDNANTLYLQMSSL RSEDALYYCARREVVVYWFYFDVWGTG TTVTVSS
241		VL	DIQMTQSPASLSVSVGETVTITCRASENIY SNLAWYQQKQKSPQLLVFSAKNLADG VPSRFGGSGSGTQYSLKINLSQSEDFGT YCYHFWGTPLTFGAGTKLELK
242	AK10	VH	EVQLQQSGPELMKPGASVKISCKASGYTF TDYYMNWVKQSHGKSLEWIGDINPKIGD TIYNQKFKGKATLTVDKSSSTAYMELRSL TSEDSAVYYCARWDYYGSSSLFDYWGQG TTLTVSS
243		VL	DIVMTQSHKFMSTSVGDRVSITCKASQDV TTAVAWYQQKPGQSPKLLIYWASTRHTG VPDRFTGTGSGTDYTLTISNVQAEDLALY YCYQHSTPYTFGGGKLEIK
244	AK09	VH	QIQLVQSGPELKKPGETVKISCKASGYTLT TYGMSWVKQAPGKGLKWMGWINTYSG VPTYADEFKGRFAFSLETSASTAYLQINN LKNEDTATYFCARLGYYGYFDYWGQG TTLTVSS
245		VL	DVVLQSPATLSVTPGDSVSLSCRASQSIR NNLHWYQQKSHESPRLLIKYIFQSISGIPS RFSGSGSGTEFTLSINSVETEDFGMYFCQQ SDSWPHVTFGAGTKLELI
246	AK08	VH	QVQLKESGPGLVAPSQSL SITCTVSGFSLT SYGVNWIRQPPGKGLEWLGVIWGDGSTN YHSALISRLSISKDNSKSQVFLKLNSLQTD DTATYYCAKALSDWYFDVWGTGTTVT SS
247		VL	QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWYQQKPGSSPKPWIYATSNLASGVP ARFSGSGSGTSYSLTISRVEAEDAATYYC QQWSSNPYTFGGGKLEIK
248	AK07	VH	EVQLQQSGPELVKPGASVKMSCKASGYT FTDYKMHVWKQSHGKSLEWIGNINPNNG GTSYNQKFKGKATLTVNKSSSTAYMELR SLTSEDSAVYYCARRYYGSSSYFDYWG QGTTTLTVSS
249		VL	QIVLTQSPAIMASASPGEKVTMTCSVSSSVS YMHWYQQKSGTSPKRWIYDTSKLASGVP ARFSGSGSGTSYSLTISSMEAEDAATYYC QQWSSNPPTFGAGTKLELK
250	AK06	VH	EVPLVESGGGLVKPGSLKLSCAASGFTF SDYGIHWVRQAPEKGLEWVAYITSVSSTI YYADTVKGRFTISRDNANTLFLQMTSLR

			SEDTAMYYCAILDWYFDVWGTGTTVTVS S
251		VL	QIVLTQSPAIMASASPGEKVTMTCSANSSVS FMHWFQQKSGTSPKRWIYDTSKLASGVP ARFSASGSGTSYSLTISSMEAEDAATYYC QQWSSNPLTFGAGTKVELK
252	AK05	VH	QVQLQQPGTEL VKPGASVQLSCKASGYIF TSYWYMWVKQRPGQGLEWIGNVNPYSG DTNYNEKFVKATLTVDKASSTAFIQLSG LTSEDSAVYFCARGDGLYYYAMDYWGQ GTSVTVSS
253		VL	DIKMTQSPSSMYASLGERVTITCKASQDI NSYLTWFQQKPGKSPKTLIYRASRLVDGV PSRFSGSGSGQDYSLTISSLEYEDMGIYYC LQYDEFPLTFGAGAKLELI
254	AK04	VH	QVQLKESGPGLVAPSQSL SITCTVSGFSLT SYGVS WVRKPPGKGLEWLGVIWHDGSTS YHSTLISRLNISRDNSKSQVFLKLNLSLQTD DTATYYCASDGYSGTFAYWGQGLTVTS A
255		VL	QIVVTQSPAIMASASLGERVTMTCTASSSVS SSYLHWYQQKPGSSPKLWIYSTSILASGV PARFSGSGSGTSYSLTVSSMEAEDAATYY CHQYHRSPYTFGGGSKLEIR
256	AK03	VH	EVQLQQSGTVLARPGASVKMSCKTSGYT FTSYWMHWVKQRPGQGLEWIGTIYPGNS DSTYNQKFKGKAKLTAVTSASTAYMEVS SLTNEDSAVYYCSREGTVVANYYGLDY WGQGTSVTVSS
257		VL	QIVLSQSPAILSASPGEKVTMTCRASSSVS YIHWYQQKPGSSPKPWIYATSNLASGVPA RFSGSGSGTSYSLTISRVEAEDAATYYCQ QWSSKPPTFGAGTKLELK
258	AK02	VH	QVQLQQPGAEL VKPGASVKLSCKASGYT FTNYIYHWVKQRPGQGLEWIGMIHPISGS TNYNEKLKIKATLTVDKSSSTAYMQLSSL TSEDSAVYYCARRYYGSSYWFYFDVWGT GTTVTVSS
259		VL	QIVLSQSPAILSASPGEKVTMTCRASSSVS YMNWYQQKPGSSPKPWIYATSNLASGVP ARFSGSGSGTPYSLTISRVEAEDAATYYC QQWSSHPPTFGSGTKLEIK
260	AK01	VH	QVQLKQSGPGLVQPSQSL SITCTVSGFSLT SYSVHWVRQSPGKGLEWLGVIWSSGGSTD YNAVFISRLSISRDNSKSQVFFKMNSLQA DDSAIYYCASYSYYTMDYWGQGTSVTVS S
261		VL	DVVMQTQPLSLPVSLGDQASISCRSSQSL VLDNGNTYLHWY LQKPGQSPKLLIYKVS NRFSGVPDRFTGSGSGTDFTLKINRVEAE DLGVYFCSQSTHVPFTFGSGTKLEIK
262	AK16	VH	EVQLQQSGAELVRPGASVKLSASGFNI KDDYMHWVKQRPGQGLEWIGWIDLENG DTEYASKFQGKATITADTSSNTAYLQLSS LTSEDTAVYYCTPIGTVAEGFAYWGQ GTLVTVSA

263		VL	DIQMTQSPASLSVSVGETVTITCRASENIY SNLAWYQQKPGKSPQLLVYAATNLADG VPSRFGSGSGTQYSLKINSLQSEDFGSYY CQHFWGTPYTFGGGKLEIK
264	AK17	VH	EVQLQQSGPELVKPGASVKMSCKASGYT FTDYKMHVVKQSHGKSLEWIGNINPNNG GTSYNQKFKGKATLTVNKSSSTAYMELR SLTSEDSAVYYCARRYYGSSSSYFDYWG QGTTTLTVSS
265		VL	DILLTQSPAILSVPGERVSFSCRASQSIGT SIHWYQQRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLINSVESEDIADYYCQQSN SWPTTFGGGKLEIK
266	AK18	VH	EELLVESGGGLVKPGGSLKLSCAASGFTF SDYGMHWVRQAPEKGLEWVAYISSDSST IYYADTVKGRFTISRDNAKNTLFLQMTSL RSEDAMYYCARTIYYGNFYFDYWGQGT TLTVSS
267		VL	QIVLTQSPAIMASAPGERVTMTCSASSSVS SSSLYWYQQKSGSSPKLWIYSMSNLASG VPAKFSGSGSGTSYSLTINSMEAEDAATY YCQQWSSNPLTFGAGTKLELK

[0165] In some embodiments, a bispecific antibody of the present disclosure may comprise an anti-Siglec-6 antigen binding domain according to any of the embodiments herein and an anti-Siglec-8 antigen binding domain according to any of the embodiments herein. In some embodiments, the bispecific antibody comprises an anti-Siglec-6 antigen binding domain comprising a VH region comprising an HVR-H1 comprising the amino acid sequence of SEQ ID NO:124, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:125, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:126 and a VL region comprising an HVR-L1 comprising the amino acid sequence of SEQ ID NO:127, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:128, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:129 and an anti-Siglec-8 antigen binding domain comprising a VH region comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63 and a VL region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66. In some embodiments, the bispecific antibody comprises an anti-Siglec-6 antigen binding domain comprising a VH region comprising an HVR-H1 comprising the amino acid sequence of SEQ ID NO:208, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:209, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:210 and a VL region comprising an

HVR-L1 comprising the amino acid sequence of SEQ ID NO:211, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:212, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:213 and an anti-Siglec-8 antigen binding domain comprising a VH region comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63 and a VL region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66. In some embodiments, the anti-Siglec-6 antigen binding domain comprises a VH region comprising the amino acid sequence of SEQ ID NO:254 and a VL region comprising the amino acid sequence of SEQ ID NO:255, and the anti-Siglec-8 antigen binding domain comprises a VH region comprising the amino acid sequence of SEQ ID NO:6 and a VL region comprising the amino acid sequence of SEQ ID NO:16 or 21. In some embodiments, the anti-Siglec-6 antigen binding domain comprises a VH region comprising the amino acid sequence of SEQ ID NO:260 and a VL region comprising the amino acid sequence of SEQ ID NO:261, and the anti-Siglec-8 antigen binding domain comprises a VH region comprising the amino acid sequence of SEQ ID NO:6 and a VL region comprising the amino acid sequence of SEQ ID NO:16 or 21.

[0166] In some embodiments, an anti-Siglec-6 antigen binding domain of the present disclosure binds to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell. In some embodiments, binding of the bispecific antibody to the extracellular domain of human Siglec-6 inhibits activation of the mast cell. Assays for assessing mast cell activation are known in the art and exemplified herein. In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 inhibits CD63 expression, *e.g.*, of a human mast cell. In some aspects, a bispecific antibody described herein inhibits one or more mast cell-mediated activities. In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 inhibits expression and/or release (*e.g.*, by a human mast cell) of one or more of the following: IL-6, IL-8, IL-13, CCL2, CCL4, histamine, chymase, and tryptase (*e.g.*, active tryptase). In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 inhibits expression and/or release (*e.g.*, by a human mast cell) of one or more of the following inflammatory mediators: proteases (*e.g.*, pan-tryptase, active or beta-tryptase, chymase, CPA3, heparin, etc.), leukotrienes (*e.g.*, leukotriene C4 or B4, platelet activating factor, prostaglandin D2 or E2, etc.), amines (*e.g.*, histamine, serotonin, dopamine, polyamines,

etc.), growth factors (*e.g.*, SCF, GM-CSF, FGF, EGF, NGF, VEGF, PDGF, etc.), cytokines (*e.g.*, TNF, IL-1b, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, IL-17, IL-18, IL-31, IL-36, etc.), chemokines (*e.g.*, CCL2, CCL3, CCL4, CCL5, CCL11, CCL12, CCL13, CCL24, CCL26, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, etc.), exosomes, and extracellular traps. For example, total and active tryptase as well as histamine, N-methyl histamine, and 11-beta-prostaglandin F2 can be measured in blood or urine to assess the reduction in mast cells. See, *e.g.*, U.S. Patent Application Publication No. US 20110293631 for an exemplary mast cell activity assay.

[0167] In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to reduced levels of Siglec-6 on the cell surface (*i.e.*, of the human mast cell). Binding of the antibody can lead to reduced levels of Siglec-6 on the cell membrane, *e.g.*, through Siglec-6 internalization, endocytosis, shedding, cleaving, etc. Assays for assessing levels of Siglec-6 surface expression are known in the art and exemplified herein. In some embodiments, Siglec-6 surface expression is measured by flow cytometry, *e.g.*, using an anti-Siglec-6 antibody with a detectable (*e.g.*, fluorescent) tag. For example, as demonstrated herein, mast cells can be contacted with an anti-Siglec-6 antibody, and surface expression can be measured by flow cytometry with a fluorescent-tagged anti-Siglec-6 antibody that binds to a different epitope on Siglec-6 than the test antibody. Reduced fluorescence in the presence of the test antibody can indicate a reduced level of Siglec-6 surface expression. In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to reduced levels of Siglec-6 surface expression regardless of the presence/absence of an antibody Fc region, or regardless of the ability of the antibody Fc region to bind an Fc receptor (*e.g.*, expressed on an effector cell). In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to an expression level Siglec-6 on the cell membrane that is reduced by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%, *e.g.*, as compared to surface expression of Siglec-6 in the absence of an antibody, or in the absence of an antibody that does not bind to Siglec-6.

[0168] In some embodiments, binding of the bispecific antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to reduced levels of Siglec-6 on the cell membrane (*i.e.*, of the human mast cell) in the presence of a cell (*e.g.*, an

effector cell) expressing an Fc receptor. In some embodiments, the antibody comprises an Fc region (*e.g.*, an active Fc region). In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to a reduced level of surface-expressed Siglec-6 dependent upon interaction between the antibody Fc region (*e.g.*, an active Fc region) and an Fc receptor or an effector cell, *e.g.*, that expresses an Fc receptor. In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to a reduced level of surface-expressed Siglec-6 only when the antibody is a full-length antibody comprising an Fc region (*e.g.*, an active Fc region), *e.g.*, in the presence of an Fc receptor or an effector cell (*e.g.*, expressing an Fc receptor). In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to a reduced level of surface-expressed Siglec-6 when the antibody is an antibody fragment, *e.g.*, lacking an Fc region. In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to a reduced level of surface-expressed Siglec-6 when the antibody is a full-length antibody comprising an Fc region that does not bind an Fc receptor (*e.g.*, an inactive or dead Fc region lacking effector function). In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to a reduced level of surface-expressed Siglec-6 when the antibody is a full-length antibody comprising an Fc region (*e.g.*, an active Fc region), *e.g.*, in the presence of an Fc receptor or an effector cell (*e.g.*, expressing an Fc receptor), but not when the antibody is an antibody fragment lacking an Fc region or comprising an Fc region that does not bind an Fc receptor (*e.g.*, an inactive or dead Fc region). In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to an expression level Siglec-6 on the cell membrane that is reduced by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100% when the antibody comprises an active Fc region, *e.g.*, as compared to surface expression of Siglec-6 using an antibody that does not comprise an Fc region, or does not comprise an active Fc region.

[0169] In some embodiments, binding of the bispecific antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell induces dimerization and/or internalization of Siglec-6. In some embodiments, binding of the antibody to the extracellular

domain of human Siglec-6 when expressed on a surface of a human mast cell induces dimerization and internalization of Siglec-6. In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell induces endocytosis of Siglec-6. In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell induces shedding of Siglec-6 (*e.g.*, the Siglec-6 ECD). In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell induces cleaving of Siglec-6 (*e.g.*, the Siglec-6 ECD).

[0170] In some embodiments, a bispecific antibody described herein depletes mast cells expressing human Siglec-6 *in vitro* and/or *in vivo*, *e.g.*, in the presence of effector cells. In some embodiments, binding of the antibody to the extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell induces antibody-dependent cellular cytotoxicity (ADCC) and/or antibody-dependent cellular phagocytosis (ADCP) activity, *e.g.*, in the presence of effector cells *in vitro* and/or *in vivo*. In some other aspects, an anti-Siglec-6 antibody described herein kills mast cells expressing Siglec-6 by ADCC activity *in vitro* and/or *in vivo*. In some other aspects, a bispecific antibody described herein induces phagocytosis of mast cells expressing Siglec-6 and/or Siglec-8 by ADCP activity *in vitro* and/or *in vivo*. In some embodiments, a composition comprises non-fucosylated (*i.e.*, afucosylated) bispecific antibodies. In some embodiments, a composition comprising non-fucosylated bispecific antibodies described herein enhances ADCC activity as compared to a composition comprising partially fucosylated bispecific antibodies.

[0171] Assays for assessing ADCC activity are well known in the art and described herein. In an exemplary assay, to measure ADCC activity, effector cells and target cells are used, *e.g.*, at a specific ratio (*e.g.*, 1:50 target cells:effector cells) in the presence of an antibody to be evaluated. Examples of effector cells include natural killer (NK) cells, large granular lymphocytes (LGL), lymphokine-activated killer (LAK) cells and PBMC comprising NK and LGL, or leukocytes having Fc receptors on the cell surfaces, such as neutrophils, eosinophils and macrophages. The target cell is any cell which expresses on the cell surface antigens that antibodies to be evaluated can recognize. An example of such a target cell is a mast cell which expresses Siglec-6 on the cell surface. Target cells are labeled with a reagent that enables detection of cytolysis. Examples of reagents for labeling include a radio-active substance such as sodium chromate ($\text{Na}_2 \text{}^{51}\text{CrO}_4$) and carboxyfluorescein succinimidyl ester. See, *e.g.*, Immunology, 14, 181 (1968);

J. Immunol. Methods, 172, 227 (1994); and J. Immunol. Methods, 184, 29 (1995). The number of remaining target cells can then be assessed (*e.g.*, by flow cytometry) after co-incubation and normalized (*e.g.*, to number of remaining target cells co-incubated with effector cells treated with an antibody that does not bind to Siglec-6 or treated with no antibody).

[0172] Assays for assessing ADCP activity are well known in the art and described herein. For example, target cells can be co-cultured with macrophages (*e.g.*, human monocyte-derived macrophages), monocytes, or PBMCs at a specific ratio (*e.g.*, 1:50 target cells:macrophages) in the presence of an antibody to be evaluated. The number of remaining target cells can then be assessed (*e.g.*, by flow cytometry) after co-incubation and normalized (*e.g.*, to number of remaining target cells co-incubated with macrophages treated with an antibody that does not bind to Siglec-6, or treated with no antibody).

[0173] In some embodiments, binding of a bispecific antibody of the present disclosure to an extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to a reduced level of Siglec-6 on the cell surface. In some embodiments, binding of a bispecific antibody of the present disclosure to an extracellular domain of human Siglec-6 inhibits activation of a mast cell that expresses the human Siglec-6.

[0174] In some embodiments, binding of a bispecific antibody of the present disclosure to an extracellular domain of human Siglec-8 when expressed on a surface of a human mast cell leads to a reduced level of Siglec-8 on the cell surface. In some embodiments, binding of a bispecific antibody of the present disclosure to an extracellular domain of human Siglec-8 when expressed on a surface of a human eosinophil leads to a reduced level of Siglec-8 on the cell surface. In some embodiments, binding of a bispecific antibody of the present disclosure to an extracellular domain of human Siglec-8 inhibits activation of a mast cell that expresses the human Siglec-8. In some embodiments, binding of a bispecific antibody of the present disclosure to an extracellular domain of human Siglec-8 when expressed on a surface of a human eosinophil depletes eosinophils expressing human Siglec-8 *in vivo*.

[0175] In some embodiments, an antigen binding domain of the present disclosure is a single-domain antigen-binding domain. A single-domain antigen-binding domain is a single polypeptide chain comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antigen-binding domain is a human single-domain antigen-binding domain (Domantis, Inc., Waltham, Mass.; see, *e.g.*, U.S. Pat. No. 6,248,516 B1). In one embodiment, a single-

domain antigen-binding domain consists of all or a portion of the heavy chain variable domain of an antigen-binding domain.

[0176] In some embodiments, amino acid sequence modification(s) of the antigen-binding domains described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

[0177] A useful method for identification of certain residues or regions of the antigen-binding domain that are preferred locations for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. Here, 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 (e.g., 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 is conducted at the target codon or region and the expressed immunoglobulins are screened for the desired activity.

[0178] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme or a polypeptide which increases the serum half-life of the antibody.

[0179] In some embodiments, monoclonal antibodies have a C-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the C-

terminus of heavy chain and/or light chain. In some embodiments, the C-terminal cleavage removes a C-terminal lysine from the heavy chain. In some embodiments, monoclonal antibodies have an N-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the N-terminus of heavy chain and/or light chain. In some embodiments, truncated forms of monoclonal antibodies can be made by recombinant techniques.

[0180] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a different residue. Sites of interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 5 under the heading of “preferred substitutions.” If such substitutions result in a desirable change in biological activity, then more substantial changes, denominated “exemplary substitutions” in Table 5, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 5.

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)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe

Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu
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[0181] Substantial modifications in the biological properties of the antibody are 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. Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)):

- (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)
- (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)
- (3) acidic: Asp (D), Glu (E)
- (4) basic: Lys (K), Arg (R), His (H)

[0182] Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

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

[0183] Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, into the remaining (non-conserved) sites.

[0184] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have modified (e.g., improved) biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g., 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibodies thus generated are displayed from filamentous phage particles as fusions to at least part of a phage coat protein (e.g., 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). In order to identify candidate hypervariable region sites for modification, scanning mutagenesis (e.g., alanine scanning) 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 and antigen. Such contact residues and neighboring residues are candidates for substitution according to techniques known in the art, including those elaborated herein. Once such variants are generated, the panel of variants is subjected to screening using techniques known in the art, including those described herein, and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0185] Nucleic acid molecules encoding amino acid sequence variants of the antibody are 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.

[0186] In one aspect, a bispecific antibody described herein is an antibody fragment (including antigen-binding fragment), *e.g.*, a Fab, Fab'-SH, Fv, scFv, or (Fab')₂ fragment. In one aspect, a bispecific antibody described herein comprises an antibody fragment (including antigen-binding fragment), *e.g.*, a Fab, Fab'-SH, Fv, scFv, or (Fab')₂ fragment. In one aspect, any of the bispecific antibodies described herein are purified.

[0187] There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α , δ , ϵ , γ and μ , respectively. The γ and α classes are further divided into subclasses *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. IgG1 antibodies can exist in multiple polymorphic variants termed allotypes (reviewed in Jefferis and Lefranc 2009. mAbs Vol 1 Issue 4 1-7) any of which are suitable for use in some of the embodiments herein. Common allotypic variants in human populations are those designated by the letters a,f,n,z or combinations thereof. In any of the embodiments herein, the bispecific antibody may comprise a heavy chain Fc region, *e.g.*, an Fc region comprising a human IgG Fc region. In further embodiments, the human IgG Fc region comprises a human IgG1 or IgG4. In some embodiments, the bispecific antibody is an IgG1 antibody. In some embodiments, the bispecific antibody is an IgG2 antibody. In some embodiments, the bispecific antibody is an IgG4 antibody. In some embodiments, the human IgG4 comprises the amino acid substitution

S228P, wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the human IgG1 comprises the amino acid sequence of SEQ ID NO:78. In some embodiments, the human IgG4 comprises the amino acid sequence of SEQ ID NO:79.

[0188] It may be desirable to introduce one or more amino acid modifications in an Fc region of antibodies of the present disclosure, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions including that of a hinge cysteine. In some embodiments, the Fc region variant comprises a human IgG4 Fc region. In a further embodiment, the human IgG4 Fc region comprises the amino acid substitution S228P, wherein the amino acid residues are numbered according to the EU index as in Kabat.

[0189] In accordance with this description and the teachings of the art, it is contemplated that in some embodiments, an antibody of the present disclosure may comprise one or more alterations as compared to the wild type counterpart antibody, e.g. in the Fc region. These antibodies would nonetheless retain substantially the same characteristics required for therapeutic utility as compared to their wild type counterpart. For example, it is thought that certain alterations can be made in the Fc region that would result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in WO99/51642. See also Duncan & Winter Nature 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO94/29351 concerning other examples of Fc region variants. WO00/42072 (Presta) and WO 2004/056312 (Lowman) describe antibody variants with improved or diminished binding to FcRs. The content of these patent publications are specifically incorporated herein by reference. See, also, Shields et al. J. Biol. Chem. 9(2): 6591-6604 (2001). In some embodiments, the antibody may comprise one or more Fc mutations that extend half-life, e.g., *in vivo*. Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). These antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in U.S. Pat. No. 6,194,551B1, WO99/51642. See, also, Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

[0190] In some embodiments, the human IgG1 Fc region comprises one or more mutation(s) that reduce effector function. In some embodiments, the human IgG1 Fc region comprises a substitution or deletion at one or more of the following position(s), numbering based on EU index: (a) L234 and/or L235; (b) A327, A330, and/or P331; (c) E233, L234, L235, and/or G236; (d) E233, L234, and/or L235; (e) E233, L234, L235, G236, A327, A330, and/or P331; (f) E233, L234, L235, A327, A330, and/or P331; (g) N297; (h) L242, N297, and/or K334; (i) A287, N297, and/or L306; (j) R292, N297, and/or V302; (k) N297, V323, and/or I332; (l) V259, N297, and/or L306; (m) L234, L235, K322, M252, S254, and/or T256; or (n) L234, L235, and/or P329. In some embodiments, the antibody comprises a human IgG1 Fc region with one or more of the following mutation(s), 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. *See, e.g.,* Schlothauer, T. *et al.* (2016) *Protein Eng. Des. Sel.* 29:457-466; Armour, K.L. *et al.* (2003) *Mol. Immunol.* 40:585-593; Jacobsen, F.W. *et al.* (2017) *J. Biol. Chem.* 292:1865-1875; and Borrok, M.J. *et al.* (2017) *J. Pharm. Sci.* 106:1008-1017. In some embodiments, the antibody heavy chain comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO:268 or 269.

[0191] In some embodiments, the human IgG1 Fc region comprises one or more mutation(s) that increase or enhance effector function. In some embodiments, the human IgG1 Fc region comprises a substitution or deletion at one or more of the following position(s), numbering based on EU index: (a) F243, R292, Y300, V305, and/or P396; (b) S239 and/or I332; (c) S239, I332, and/or A330; (d) S298, E333, and/or K334; (e) G236, S239, and/or I332; (f) K326 and/or E333; (g) S267, H268, and/or S324; or (h) E345, E430, and/or S440. In some embodiments, the human IgG1 Fc region comprises one or more of the following mutation(s), 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. *See, e.g.,* Stavenhagen, J.B. *et al.* (2007) *Cancer Res.* 67:8882-8890; Lazar, G.A.

et al. (2006) *Proc. Natl. Acad. Sci. USA* 103:4005-4010; Shields, R.L. *et al.* (2001) *J. Biol. Chem.* 276:6591-6604; Richards, J.O. *et al.* (2008) *Mol. Cancer Ther.* 7:2517-2527; Idusogie, E.E. *et al.* (2001) *J. Immunol.* 166:2571-2575; Moore, G.L. *et al.* (2010) *MAbs* 2:181-189; and Diebolder, C.A. *et al.* (2014) *Science* 343:1260-1263.

[0192] In some embodiments, the human IgG2 Fc region comprises one or more mutation(s) that reduce effector function. In some embodiments, the human IgG2 Fc region comprises a substitution or deletion at one or more of the following position(s), numbering based on EU index: (a) A330 and/or P331; (b) V234, G237, P238, H268, V309, A330, and/or P331; or (c) V234, G237, H268, V309, A330, P331, C232, C233, S267, L328, M252, S254, and/or T256. In some embodiments, the human IgG2 Fc region comprises one or more of the following mutation(s), 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. *See, e.g.,* Armour, K.L. *et al.* (2003) *Mol. Immunol.* 40:585-593 and US PG Pub. Nos. 20170204193 and 20170240631.

[0193] In some embodiments, the human IgG4 Fc region comprises one or more mutation(s) that reduce effector function. In some embodiments, the human IgG4 Fc region comprises a substitution or deletion at one or more of the following position(s), numbering based on EU index: (a) E233, F234, L235, and/or G236; (b) E233, F234, and/or L235; or (c) S228 and/or L235. In some embodiments, the human IgG4 Fc region comprises one or more of the following mutation(s), 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. *See, e.g.,* Schlothauer, T. *et al.* (2016) *Protein Eng. Des. Sel.* 29:457-466; and Armour, K.L. *et al.* (2003) *Mol. Immunol.* 40:585-593. In some embodiments, the antibody heavy chain comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO:270.

[0194] In some embodiments, the bispecific antibody of the present disclosure comprises an antibody heavy chain comprising a heavy chain constant region that comprises an amino acid sequence shown in Table 8.

Table 8. Antibody heavy chain constant region sequences.

SEQ ID NO	Sequence Description	Sequence
268	Human IgG1 constant region (f allotype)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL

		MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPG
269	Human IgG1 constant region (z allotype)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPG
270	Human IgG4 S228P constant region	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNV PSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLG

[0195] In some embodiments, a bispecific antibody of the present disclosure comprises an antibody light chain comprising a light chain constant (CL) domain, *e.g.*, a human kappa or lambda CL domain. In some embodiments, the CL domain is a human kappa CL domain. In some embodiments, the CL domain comprises the amino acid sequence of RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:271).

[0196] A variety of formats suitable for bispecific antibodies are known in the art and contemplated for use herein; exemplary and non-limiting formats are described in greater detail *infra*. In some embodiments, the bispecific antibody comprises a first antibody heavy chain comprising a first VH region and a first Fc region, a first antibody light chain comprising a first VL region, a second antibody heavy chain comprising a second VH region and a second Fc region, and a second antibody light chain comprising a second VL region; wherein the first VH and VL regions form the first antigen binding domain; and wherein the second VH and VL regions form the second antigen binding domain. In some embodiments, one or both heavy chain-light chain pairs comprises a modification to favor proper heavy chain-light chain pairing, *e.g.*, favoring association of the particular heavy and light chain forming one of the antigen binding domains and/or disfavoring mispairing between heavy and light chains (*e.g.*, a heavy

chain belonging to one antigen binding domain paired with a light chain belonging to a different antigen binding domain). In some embodiments, one or both heavy chains comprises a modification to favor heterodimerization of the heavy chains (*e.g.*, association of one arm comprising one antigen binding domain and another arm comprising a different antigen binding domain) and/or disfavor homodimerization of the heavy chains (*e.g.*, association of two arms comprising the same antigen binding domain). As used herein, an “arm” of a bispecific antibody may refer to a half-antibody comprising the antibody heavy chain and antibody light chain that makes up one of the antigen binding domains of the present disclosure, *e.g.*, in which the VH region of the heavy chain and VL region of the light chain form an anti-Siglec-6 or anti-Siglec-8 antigen binding domain of the present disclosure. In some embodiments, a bispecific antibody of the present disclosure comprises two arms: one forming an anti-Siglec-6 antigen binding domain of the present disclosure, and the other forming an anti-Siglec-8 antigen binding domain of the present disclosure.

[0197] One such format has been described as the DuetMab format (Medimmune LLC). *See, e.g.*, U.S. Pat. No. 9,527,927. In this format, one or more interchain cysteines have been relocated which, in some aspects, results in the relocation of an interchain disulphide linkage at the HC-LC interface. In some aspects, this involves modification of one heavy chain and the corresponding light chain within an antibody whereby a native cysteine is substituted by a non-cysteine amino acid, and a native non-cysteine amino acid is substituted by a cysteine amino acid. In some aspects, the antibodies provided herein are bispecific which means they contain variable domains with different antigen and/or epitope specificities. In certain aspects, the modified heavy and light chain duplex for a given antibody contains a variable domain with a certain antigen specificity and the unmodified heavy and light chain duplex within the same antibody contains a variable domain with a different antigen specificity. Another format with engineered disulfide bonds between heavy and light chains is the BIClonal format; *see, e.g.*, Litvak-Greenfeld, D. *et al.* (2019) *Methods Mol Biol* 1904:431-454.

[0198] In some embodiments, one of the antibody heavy chains comprises a first constant heavy chain (CH1) region that comprises a substitution of a native cysteine to a non-cysteine amino acid and a substitution of a native non-cysteine amino acid to a cysteine amino acid, and the corresponding antibody light chain comprises a constant light chain (CL) region that comprises a substitution of a native cysteine to a non-cysteine amino acid and a substitution of a native non-cysteine amino acid to a cysteine amino acid, such that the substituted cysteines on

the CH1 region and the CL region on the corresponding heavy and light chains form a disulfide bond. In some embodiments, the other antibody heavy chain and antibody light chain do not contain said substitutions. In some embodiments, the arm comprising the anti-Siglec-6 antigen binding domain comprises the substituted CH1 and CL regions, and the arm comprising the anti-Siglec-8 antigen binding domain does not. In some embodiments, the arm comprising the anti-Siglec-8 antigen binding domain comprises the substituted CH1 and CL regions, and the arm comprising the anti-Siglec-6 antigen binding domain does not. An example of this format is provided in **FIG. 1A**.

[0199] In some embodiments, the substituted CH1 region comprises an F126C substitution, and the substituted CL region comprises an S121C substitution. In some embodiments, the substituted CH1 region further comprises a C220V substitution, and the substituted CL region further comprises a C214V substitution.

[0200] Another such format has been described as the CrossMab format (Roche). *See, e.g.*, International Publication No. WO2009080253. In this format, the CH1 and CL regions in one arm are replaced by each other, leaving the CH1 region in the light chain and the CL region in the heavy chain, while the other arm retains its native configuration. In some embodiments, the ratio of a desired bivalent, bispecific antibody compared to undesired side products can be improved by the replacement of certain domains in only one pair of heavy chain and light chain (HC/LC). While the first of the two HC/LC pairs originates from an antibody specifically binding to a first antigen and is left essentially unchanged, the second of the two HC/LC pairs originates from an antibody specifically binding to a second antigen, and is altered by the following replacement: light chain: replacement of the constant light chain domain CL by the constant heavy chain domain CH1 of said antibody specifically binding to a second antigen, and heavy chain: replacement of the constant heavy chain domain CH1 by the constant light chain domain CL of said antibody specifically binding to a second antigen. Thus the resulting bivalent, bispecific antibodies are artificial antibodies which comprise a) the light chain and heavy chain of an antibody specifically binding to a first antigen; and b) the light chain and heavy chain of an antibody specifically binding to a second antigen; wherein said light chain (of an antibody specifically binding to a second antigen) contains a constant domain CH1 instead of CL wherein said heavy chain (of an antibody specifically binding to a second antigen) contains a constant domain CL instead of CH1.

[0201] In some embodiments, the arm comprising the anti-Siglec-6 antigen binding domain comprises an antibody heavy chain that comprises a constant light chain (CL) region that replaces a native first constant heavy chain (CH1) region and an antibody light chain that comprises a CH1 region that replaces a native CL region, and the arm comprising the anti-Siglec-8 does not. In some embodiments, the arm comprising the anti-Siglec-8 antigen binding domain comprises an antibody heavy chain that comprises a constant light chain (CL) region that replaces a native first constant heavy chain (CH1) region and an antibody light chain that comprises a CH1 region that replaces a native CL region, and the arm comprising the anti-Siglec-6 does not. Examples of these formats are illustrated in **FIG. 1B**.

[0202] In another format, the VH and VL regions in one arm are replaced by each other, leaving the VH region in the light chain and the VL region in the heavy chain, while the other arm retains its native configuration. In addition, engineered electrostatic interactions between the CH1 and VL regions can be introduced, such that the CH1 region of one arm can include substitution of one or more native, non-positively charged amino acid(s) to positively charged amino acid(s), and the corresponding CL region of the same arm can include substitution of one or more native, non-negatively charged amino acid(s) to negatively charged amino acid(s) and/or the CL region of the other arm can include substitution of one or more native, non-positively charged amino acid(s) to positively charged amino acid(s), and the corresponding CH1 region of the same arm can include substitution of one or more native, non-negatively charged amino acid(s) to negatively charged amino acid(s). Examples of these formats are illustrated in **FIG. 1C**.

[0203] In some embodiments, the light chain on one arm comprises a Q124E, while the heavy chain on the other arm comprises K147E and K213E substitutions and its corresponding light chain comprises E123K and Q124K substitutions.

[0204] One approach known in the art for making bispecific antibodies is the “knobs-into-holes” or “protuberance-into-cavity” approach (see, *e.g.*, US Pat. No. 5,731,168 and Ridgway, J.B. *et al.* (1996) *Protein Eng* 9(7):617-621). In this approach, two immunoglobulin polypeptides (*e.g.*, heavy chain polypeptides) each comprise an interface. An interface of one immunoglobulin polypeptide (*e.g.*, the heavy chain of one arm of bispecific antibody of the present disclosure) interacts with a corresponding interface on the other immunoglobulin polypeptide (*e.g.*, the heavy chain of the other arm of bispecific antibody of the present disclosure), thereby allowing the two immunoglobulin polypeptides to associate in an

assembled, heterodimeric bispecific antibody. These interfaces may be engineered such that a “knob” or “protuberance” (these terms may be used interchangeably herein) located in the interface of one immunoglobulin polypeptide corresponds with a “hole” or “cavity” (these terms may be used interchangeably herein) located in the interface of the other immunoglobulin polypeptide. In some embodiments, the hole is of identical or similar size to the knob and suitably positioned such that when the two interfaces interact, the knob of one interface is positionable in the corresponding hole of the other interface. Without wishing to be bound to theory, this is thought to stabilize the heteromultimer and favor formation of the heteromultimer over other species, for example homomultimers. In some embodiments, this approach may be used to promote the heteromultimerization of two different immunoglobulin polypeptides, creating a bispecific antibody comprising two immunoglobulin polypeptides with binding specificities for different epitopes. Examples of this approach are shown in **FIGS. 1A-1C**.

[0205] In some embodiments, a knob may be constructed by replacing a small amino acid side chain with a larger side chain. In some embodiments, a hole may be constructed by replacing a large amino acid side chain with a smaller side chain. Knobs or holes may exist in the original interface, or they may be introduced synthetically. For example, knobs or holes may be introduced synthetically by altering the nucleic acid sequence encoding the interface to replace at least one “original” amino acid residue with at least one “import” amino acid residue. Methods for altering nucleic acid sequences may include standard molecular biology techniques well known in the art. The side chain volumes of various amino acid residues are shown in the following table. In some embodiments, original residues have a small side chain volume (*e.g.*, alanine, asparagine, aspartic acid, glycine, serine, threonine, or valine), and import residues for forming a knob are naturally occurring amino acids and may include arginine, phenylalanine, tyrosine, and tryptophan. In some embodiments, original residues have a large side chain volume (*e.g.*, arginine, phenylalanine, tyrosine, and tryptophan), and import residues for forming a hole are naturally occurring amino acids and may include alanine, serine, threonine, and valine.

[0206] In some embodiments, one arm of the bispecific antibody comprises a heavy chain comprising T366W and S354C substitutions, and the other arm of the bispecific antibody comprises a heavy chain comprising Y349C, T366S, L368A, and Y407V (numbering according to EU index).

[0207] In certain embodiments, a bispecific antibody of the present disclosure is altered to increase or decrease the extent to which the antibody is glycosylated. Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of a 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 the recognition sequences for enzymatic attachment of the carbohydrate moiety to the 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-acetylglucosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0208] Addition or deletion of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that one or more of the above-described tripeptide sequences (for N-linked glycosylation sites) is created or removed. The alteration may also be made by the addition, deletion, or substitution of one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0209] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 (Presta, L.). See also US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO 2003/011878, Jean-Mairet et al. and U.S. Pat. No. 6,602,684, Umana et al. Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO 1997/30087, Patel et al. See, also, WO 1998/58964 (Raju, S.) and WO 1999/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof. See also US 2005/0123546 (Umana et al.) on antigen-binding molecules with modified glycosylation.

[0210] In certain embodiments, a glycosylation variant comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to “defucosylated” or “fucose-deficient” antibodies include: US 2003/0157108; WO 2000/61739;

WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; Okazaki et al. J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004)), and cells overexpressing β 1,4-N-acetylglycosaminyltransferase III (GnT-III) and Golgi μ -mannosidase II (ManII).

[0211] Antibodies are contemplated herein that have reduced fucose relative to the amount of fucose on the same antibody produced in a wild-type CHO cell. For example, the antibody has a lower amount of fucose than it would otherwise have if produced by native CHO cells (e.g., a CHO cell that produce a native glycosylation pattern, such as, a CHO cell containing a native FUT8 gene). In certain embodiments, an anti-Siglec-8 antibody provided herein is one wherein less than about 50%, 40%, 30%, 20%, 10%, 5% or 1% of the N-linked glycans thereon comprise fucose. In certain embodiments, an anti-Siglec-8 antibody provided herein is one wherein none of the N-linked glycans thereon comprise fucose, i.e., wherein the antibody is completely without fucose, or has no fucose or is non-fucosylated or is afucosylated. The amount of fucose can be determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn297 (e.g., complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about $\pm$ 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. In some embodiments, at least one or two of the heavy chains of the antibody is non-fucosylated.

[0212] In one embodiment, the antibody is altered to improve its serum half-life. To increase the serum half-life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Pat. No. 5,739,277, for example. 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 (US 2003/0190311, U.S. Pat. No. 6,821,505; U.S. Pat. No. 6,165,745; U.S. Pat. No. 5,624,821; U.S. Pat. No. 5,648,260; U.S. Pat. No. 6,165,745; U.S. Pat. No. 5,834,597).

[0213] The bispecific antibody described herein is prepared using techniques available in the art for generating bispecific antibodies, exemplary methods of which are described in more detail *infra*. Bispecific antibodies are monoclonal antibodies that have binding specificities for at least two different antigens. In certain embodiments, bispecific antibodies are human or humanized antibodies. In certain embodiments, one of the binding specificities is for Siglec-8 and the other is for Siglec-6. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express Siglec-8 and/or Siglec-6. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')₂ bispecific antibodies).

[0214] Methods for making bispecific antibodies are known in the art. See Milstein and Cuello, *Nature*, 305: 537 (1983), WO 93/08829 published May 13, 1993, and Traunecker et al., *EMBO J.*, 10: 3655 (1991). For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 121:210 (1986). Bispecific antibodies include cross-linked or “heteroconjugate” antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Heteroconjugate antibodies may be made using any convenient cross-linking method. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

[0215] In some embodiments, all four polypeptides comprising a bispecific antibody are produced in the same cell. In some embodiments, the heavy chain and light chain forming one arm are produced in one cell, the heavy chain and light chain forming the other arm are produced in a different cell, and the resulting arms are purified separately, then joined, e.g., via reduction.

[0216] In some embodiments, polynucleotides encoding modified immunoglobulin polypeptides with one or more corresponding knob- or hole-forming mutations may be expressed and purified using standard recombinant techniques and cell systems known in the art. See, e.g., U.S. Pat. Nos. 5,731,168; 5,807,706; 5,821,333; 7,642,228; 7,695,936; 8,216,805; U.S. Pub. No. 2013/0089553; and Spiess et al., *Nature Biotechnology* 31: 753-758, 2013. Modified immunoglobulin polypeptides may be produced using prokaryotic host cells, such as *E. coli*, or eukaryotic host cells, such as CHO cells. Corresponding knob- and hole-bearing

immunoglobulin polypeptides may be expressed in host cells in co-culture and purified together as a heteromultimer, or they may be expressed in single cultures, separately purified, and assembled *in vitro*. In some embodiments, two strains of bacterial host cells (one expressing an immunoglobulin polypeptide with a knob, and the other expressing an immunoglobulin polypeptide with a hole) are co-cultured using standard bacterial culturing techniques known in the art. In some embodiments, the two strains may be mixed in a specific ratio, *e.g.*, so as to achieve equal expression levels in culture. In some embodiments, the two strains may be mixed in a 50:50, 60:40, or 70:30 ratio. After polypeptide expression, the cells may be lysed together, and protein may be extracted. Standard techniques known in the art that allow for measuring the abundance of homo-multimeric vs. hetero-multimeric species may include size exclusion chromatography. In some embodiments, each modified immunoglobulin polypeptide is expressed separately using standard recombinant techniques, and they may be assembled together *in vitro*. Assembly may be achieved, for example, by purifying each modified immunoglobulin polypeptide, mixing and incubating them together in equal mass, reducing disulfides (*e.g.*, by treating with dithiothreitol), concentrating, and reoxidizing the polypeptides. Formed bispecific antibodies may be purified using standard techniques including cation-exchange chromatography and measured using standard techniques including size exclusion chromatography. For a more detailed description of these methods, see Speiss et al., *Nat Biotechnol* 31:753-8, 2013. In some embodiments, modified immunoglobulin polypeptides may be expressed separately in CHO cells and assembled *in vitro* using the methods described above.

[0217] In some embodiments, one of the two arms of the bispecific antibody comprises a heavy chain that comprises H435R and Y436F substitutions (numbering according to EU index). When introduced into one of the two arms of a bispecific antibody, these substitutions can allow for easier purification of the correctly assembled, heterodimeric bispecific antibody over homodimeric forms using protein A affinity chromatography; *see, e.g.*, Tustian, A. *et al.* (2016) *MAbs* 8(4):828-838.

[0218] In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:272, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:273, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:275, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:276. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid

sequence of SEQ ID NO:281, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:282, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:278, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:279. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:284, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:285, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:287, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:288. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:293, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:294, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:290, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:291. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:284, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:285, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:298, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:299.

[0219] In some embodiments, one or both of the antibody Fc domains of a bispecific antibody of the present disclosure do not have a C-terminal lysine. As is known in the art, the C-terminal lysine of some antibody heavy chain species may be cleaved off in some fraction of molecules. Therefore, in some embodiments, the present disclosure provides a composition comprising a plurality of bispecific antibodies of the present disclosure (*e.g.*, in a mixture), wherein each bispecific antibody of the plurality comprises two antibody light chains and two antibody heavy chains, and wherein the antibody heavy chains of the composition comprise some with and some without the C-terminal lysine. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:272, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:273 or 274, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:275, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:276 or 277. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:281, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:282 or 283, a second antibody light chain

comprising the amino acid sequence of SEQ ID NO:278, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:279 or 280. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:284, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:285 or 286, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:287, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:288 or 289. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:293, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:294 or 295, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:290, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:291 or 292. In some embodiments, provided herein is a bispecific antibody comprising a first antibody light chain comprising the amino acid sequence of SEQ ID NO:284, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:285 or 286, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:298, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:299 or 300.

[0220] For recombinant production of a bispecific antibody of the present disclosure, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, host cells are of either prokaryotic or eukaryotic (generally mammalian) origin. It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species.

Generating Antibodies Using Prokaryotic Host Cells:

a) Vector Construction

[0221] Polynucleotide sequences encoding polypeptide components of the antibody of the present disclosure can be obtained using standard recombinant techniques. Desired polynucleotide sequences may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer

or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic hosts. Many vectors that are available and known in the art can be used for the purpose of the present disclosure. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components, depending on its function (amplification or expression of heterologous polynucleotide, or both) and its compatibility with the particular host cell in which it resides. The vector components generally include, but are not limited to: an origin of replication, a selection marker gene, a promoter, a ribosome binding site (RBS), a signal sequence, the heterologous nucleic acid insert and a transcription termination sequence.

[0222] In some embodiments, a single polynucleotide encodes all 4 polypeptide chains for a bispecific antibody of the present disclosure. In some embodiments, a single polynucleotide encodes the heavy chain and light chain of one arm, and another polynucleotide encodes the heavy chain and light chain of the other arm. In some embodiments, each of the 4 polypeptide chains for the bispecific antibody are encoded by a separate polynucleotide. In some embodiments, provided herein is a kit comprising two polynucleotides, each encoding an arm (*e.g.*, encoding a heavy chain polypeptide and corresponding light chain polypeptide) of a bispecific antibody of the present disclosure. In some embodiments, provided herein is a kit comprising four polynucleotides, each encoding one of the four polypeptides of a bispecific antibody of the present disclosure.

[0223] In some embodiments, a single vector encodes all 4 polypeptide chains for a bispecific antibody of the present disclosure. In some embodiments, a single vector encodes the heavy chain and light chain of one arm, and another vector encodes the heavy chain and light chain of the other arm. In some embodiments, each of the 4 polypeptide chains for the bispecific antibody are encoded by a separate vector. In some embodiments, provided herein is a kit comprising two vectors, each encoding an arm (*e.g.*, encoding a heavy chain polypeptide and corresponding light chain polypeptide) of a bispecific antibody of the present disclosure. In some embodiments, provided herein is a kit comprising four vectors, each encoding one of the four polypeptides of a bispecific antibody of the present disclosure.

[0224] In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The

vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species. pBR322 contains genes-encoding ampicillin (Amp) and tetracycline (Tet) resistance and thus provides easy means for identifying transformed cells. pBR322, its derivatives, or other microbial plasmids or bacteriophage may also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of endogenous proteins. Examples of pBR322 derivatives used for expression of particular antibodies are described in detail in Carter et al., U.S. Pat. No. 5,648,237.

[0225] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, bacteriophage such as λ GEM.TM.-11 may be utilized in making a recombinant vector which can be used to transform susceptible host cells such as *E. coli* LE392.

[0226] The expression vector of the present disclosure may comprise two or more promoter-cistron pairs, encoding each of the polypeptide components. A promoter is an untranslated regulatory sequence located upstream (5') to a cistron that modulates its expression. Prokaryotic promoters typically fall into two classes, inducible and constitutive. Inducible promoter is a promoter that initiates increased levels of transcription of the cistron under its control in response to changes in the culture condition, e.g. the presence or absence of a nutrient or a change in temperature.

[0227] A large number of promoters recognized by a variety of potential host cells are well known. The selected promoter can be operably linked to cistron DNA encoding the light or heavy chain by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter sequence into the vector of the present disclosure. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes. In some embodiments, heterologous promoters are utilized, as they generally permit greater transcription and higher yields of expressed target gene as compared to the native target polypeptide promoter.

[0228] Promoters suitable for use with prokaryotic hosts include the PhoA promoter, the β -galactamase and lactose promoter systems, a tryptophan (trp) promoter system and hybrid promoters such as the tac or the trc promoter. However, other promoters that are functional in bacteria (such as other known bacterial or phage promoters) are suitable as well. Their

nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to cistrons encoding the target light and heavy chains (Siebenlist et al. (1980) Cell 20: 269) using linkers or adaptors to supply any required restriction sites.

[0229] In one aspect of the present disclosure, each cistron within the recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptides across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. The signal sequence selected for the purpose of the present disclosure should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the signal sequences native to the heterologous polypeptides, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group consisting of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA and MBP. In one embodiment of the present disclosure, the signal sequences used in both cistrons of the expression system are STII signal sequences or variants thereof.

[0230] In another aspect, the production of the immunoglobulins according to the present disclosure can occur in the cytoplasm of the host cell, and therefore does not require the presence of secretion signal sequences within each cistron. In that regard, immunoglobulin light and heavy chains are expressed, folded and assembled to form functional immunoglobulins within the cytoplasm. Certain host strains (e.g., the E. coli trxB-strains) provide cytoplasm conditions that are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits. Proba and Pluckthun Gene, 159:203 (1995).

[0231] Antibodies of the present disclosure can also be produced by using an expression system in which the quantitative ratio of expressed polypeptide components can be modulated in order to maximize the yield of secreted and properly assembled antibodies of the present disclosure. Such modulation is accomplished at least in part by simultaneously modulating translational strengths for the polypeptide components.

[0232] One technique for modulating translational strength is disclosed in Simmons et al., U.S. Pat. No. 5,840,523. It utilizes variants of the translational initiation region (TIR) within a cistron. For a given TIR, a series of amino acid or nucleic acid sequence variants can be created with a range of translational strengths, thereby providing a convenient means by which to adjust this factor for the desired expression level of the specific chain. TIR variants can be generated by

conventional mutagenesis techniques that result in codon changes which can alter the amino acid sequence. In certain embodiments, changes in the nucleotide sequence are silent. Alterations in the TIR can include, for example, alterations in the number or spacing of Shine-Dalgarno sequences, along with alterations in the signal sequence. One method for generating mutant signal sequences is the generation of a “codon bank” at the beginning of a coding sequence that does not change the amino acid sequence of the signal sequence (i.e., the changes are silent). This can be accomplished by changing the third nucleotide position of each codon; additionally, some amino acids, such as leucine, serine, and arginine, have multiple first and second positions that can add complexity in making the bank. This method of mutagenesis is described in detail in Yansura et al. (1992) *METHODS: A Companion to Methods in Enzymol.* 4:151-158.

[0233] In one embodiment, a set of vectors is generated with a range of TIR strengths for each cistron therein. This limited set provides a comparison of expression levels of each chain as well as the yield of the desired antibody products under various TIR strength combinations. TIR strengths can be determined by quantifying the expression level of a reporter gene as described in detail in Simmons et al. U.S. Pat. No. 5,840,523. Based on the translational strength comparison, the desired individual TIRs are selected to be combined in the expression vector constructs of the present disclosure.

[0234] Prokaryotic host cells suitable for expressing antibodies of the present disclosure include Archaeobacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include *Escherichia* (e.g., *E. coli*), *Bacilli* (e.g., *B. subtilis*), *Enterobacteria*, *Pseudomonas* species (e.g., *P. aeruginosa*), *Salmonella typhimurium*, *Serratia marcescans*, *Klebsiella*, *Proteus*, *Shigella*, *Rhizobia*, *Vitreoscilla*, or *Paracoccus*. In one embodiment, gram-negative cells are used. In one embodiment, *E. coli* cells are used as hosts for the present disclosure. Examples of *E. coli* strains include strain W3110 (Bachmann, *Cellular and Molecular Biology*, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), pp. 1190-1219; ATCC Deposit No. 27,325) and derivatives thereof, including strain 33D3 having genotype W3110 Δ fhuA (Δ tonA) ptr3 lac Iq lacL8 Δ ompTA(nmpc-fepE) degP41 kanR (U.S. Pat. No. 5,639,635). Other strains and derivatives thereof, such as *E. coli* 294 (ATCC 31,446), *E. coli* B, *E. coli* λ 1776 (ATCC 31,537) and *E. coli* RV308 (ATCC 31,608) are also suitable. These examples are illustrative rather than limiting. Methods for constructing derivatives of any of the above-mentioned bacteria having defined genotypes are known in the art and described in, for example, Bass et al., *Proteins*, 8:309-314 (1990). It is generally

necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, *E. coli*, *Serratia*, or *Salmonella* species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon. Typically the host cell should secrete minimal amounts of proteolytic enzymes, and additional protease inhibitors may desirably be incorporated in the cell culture.

b) Antibody Production

[0235] Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[0236] Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

[0237] Prokaryotic cells used to produce the polypeptides of the present disclosure are grown in media known in the art and suitable for culture of the selected host cells. Examples of suitable media include luria broth (LB) plus necessary nutrient supplements. In some embodiments, the media also contains a selection agent, chosen based on the construction of the expression vector, to selectively permit growth of prokaryotic cells containing the expression vector. For example, ampicillin is added to media for growth of cells expressing ampicillin resistant gene.

[0238] Any necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. Optionally the culture medium may contain one or more reducing agents selected from the group consisting of glutathione, cysteine, cystamine, thioglycollate, dithioerythritol and dithiothreitol.

[0239] The prokaryotic host cells are cultured at suitable temperatures. In certain embodiments, for *E. coli* growth, growth temperatures range from about 20° C. to about 39° C.; from about 25° C. to about 37° C.; or about 30° C. The pH of the medium may be any pH ranging from about 5 to about 9, depending mainly on the host organism. In certain embodiments, for *E. coli*, the pH is from about 6.8 to about 7.4, or about 7.0.

[0240] If an inducible promoter is used in the expression vector of the present disclosure, protein expression is induced under conditions suitable for the activation of the promoter. In one aspect of the present disclosure, PhoA promoters are used for controlling transcription of the polypeptides. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. In certain embodiments, the phosphate-limiting medium is the C.R.A.P. medium (see, e.g., Simmons et al., *J. Immunol. Methods* (2002), 263:133-147). A variety of other inducers may be used, according to the vector construct employed, as is known in the art.

[0241] In one embodiment, the expressed polypeptides of the present disclosure are secreted into and recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism, generally by such means as osmotic shock, sonication or lysis. Once cells are disrupted, cell debris or whole cells may be removed by centrifugation or filtration. The proteins may be further purified, for example, by affinity resin chromatography. Alternatively, proteins can be transported into the culture media and isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides can be further isolated and identified using commonly known methods such as polyacrylamide gel electrophoresis (PAGE) and Western blot assay.

[0242] In one aspect of the present disclosure, antibody production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant proteins. Large-scale fermentations have at least 1000 liters of capacity, and in certain embodiments, about 1,000 to 100,000 liters of capacity. These fermentors use agitator impellers to distribute oxygen and nutrients, especially glucose. Small scale fermentation refers generally to fermentation in a fermentor that is no more than approximately 100 liters in volumetric capacity, and can range from about 1 liter to about 100 liters.

[0243] In a fermentation process, induction of protein expression is typically initiated after the cells have been grown under suitable conditions to a desired density, e.g., an OD550 of about 180-220, at which stage the cells are in the early stationary phase. A variety of inducers may be used, according to the vector construct employed, as is known in the art and described above. Cells may be grown for shorter periods prior to induction. Cells are usually induced for about 12-50 hours, although longer or shorter induction time may be used.

[0244] To improve the production yield and quality of the polypeptides of the present disclosure, various fermentation conditions can be modified. For example, to improve the proper assembly and folding of the secreted antibody polypeptides, additional vectors overexpressing chaperone proteins, such as Dsb proteins (DsbA, DsbB, DsbC, DsbD and or DsbG) or FkpA (a peptidylprolyl cis,trans-isomerase with chaperone activity) can be used to co-transform the host prokaryotic cells. The chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen et al. (1999) J. Biol. Chem. 274:19601-19605; Georgiou et al., U.S. Pat. No. 6,083,715; Georgiou et al., U.S. Pat. No. 6,027,888; Bothmann and Pluckthun (2000) J. Biol. Chem. 275:17100-17105; Ramm and Pluckthun (2000) J. Biol. Chem. 275:17106-17113; Arie et al. (2001) Mol. Microbiol. 39:199-210.

[0245] To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present disclosure. For example, host cell strains may be modified to effect genetic mutation(s) in the genes encoding known bacterial proteases such as Protease III, OmpT, DegP, Tsp, Protease I, Protease Mi, Protease V, Protease VI and combinations thereof. Some E. coli protease-deficient strains are available and described in, for example, Joly et al. (1998), supra; Georgiou et al., U.S. Pat. No. 5,264,365; Georgiou et al., U.S. Pat. No. 5,508,192; Hara et al., Microbial Drug Resistance, 2:63-72 (1996).

[0246] In one embodiment, E. coli strains deficient for proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins are used as host cells in the expression system of the present disclosure.

c) Antibody Purification

[0247] In one embodiment, the antibody protein produced herein is further purified to obtain preparations that are substantially homogeneous for further assays and uses. Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, Sephadex G-75.

[0248] In one aspect, Protein A immobilized on a solid phase is used for immunoaffinity purification of the antibody products of the present disclosure. Protein A is a 41 kD cell wall

protein from *Staphylococcus aureus* which binds with a high affinity to the Fc region of antibodies. Lindmark et al (1983) J. Immunol. Meth. 62:1-13. The solid phase to which Protein A is immobilized can be a column comprising a glass or silica surface, or a controlled pore glass column or a silicic acid column. In some applications, the column is coated with a reagent, such as glycerol, to possibly prevent nonspecific adherence of contaminants.

[0249] As the first step of purification, a preparation derived from the cell culture as described above can be applied onto a Protein A immobilized solid phase to allow specific binding of the antibody of interest to Protein A. The solid phase would then be washed to remove contaminants non-specifically bound to the solid phase. Finally the antibody of interest is recovered from the solid phase by elution.

Generating Antibodies Using Eukaryotic Host Cells:

[0250] A vector for use in a eukaryotic host cell generally includes one or more of the following non-limiting components: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

a) Signal Sequence Component

[0251] A vector for use in a eukaryotic host cell may also contain a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide of interest. The heterologous signal sequence selected may be one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available. The DNA for such a precursor region is ligated in reading frame to DNA encoding the antibody.

b) Origin of Replication

[0252] Generally, an origin of replication component is not needed for mammalian expression vectors. For example, the SV40 origin may typically be used only because it contains the early promoter.

c) Selection Gene Component

[0253] Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, where relevant, or (c) supply critical nutrients not available from complex media.

[0254] One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

[0255] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

[0256] For example, in some embodiments, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. In some embodiments, an appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (e.g., ATCC CRL-9096).

[0257] Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding an antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199. Host cells may include NS0, CHOK1, CHOK1SV or derivatives, including cell lines deficient in glutamine synthetase (GS). Methods for the use of GS as a selectable marker for mammalian cells are described in U.S. Pat. No. 5,122,464 and U.S. Pat. No. 5,891,693.

d) Promoter Component

[0258] Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to nucleic acid encoding a polypeptide of interest (e.g., an antibody). Promoter sequences are known for eukaryotes. For example, virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. In certain embodiments, any or all of these sequences may be suitably inserted into eukaryotic expression vectors.

[0259] Transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[0260] The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Reyes et al., *Nature* 297:598-601 (1982), describing expression of human β -interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

e) Enhancer Element Component

[0261] Transcription of DNA encoding an antibody of the present disclosure by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the human cytomegalovirus early promoter enhancer, the mouse cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature* 297:17-18 (1982) describing enhancer elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody polypeptide-encoding sequence, but is generally located at a site 5' from the promoter.

f) Transcription Termination Component

[0262] Expression vectors used in eukaryotic host cells may also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding an antibody. One useful transcription

termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

g) Selection and Transformation of Host Cells

[0263] Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; CHOK1 cells, CHOK1SV cells or derivatives and a human hepatoma line (Hep G2).

[0264] Host cells are transformed with the above-described-expression or cloning vectors for antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

h) Culturing the Host Cells

[0265] The host cells used to produce an antibody of the present disclosure may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem. 102:255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and

thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

i) Purification of Antibody

[0266] When using recombinant techniques, the antibody can be produced intracellularly, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, may be removed, for example, by centrifugation or ultrafiltration. Where the antibody is secreted into the medium, supernatants from such expression systems may be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis, and antibiotics may be included to prevent the growth of adventitious contaminants.

[0267] The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being a convenient technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human $\gamma 1$, $\gamma 2$, or $\gamma 4$ heavy chains (Lindmark et al., J. Immunol. Methods 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human $\gamma 3$ (Guss et al., EMBO J. 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached may be agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrene-divinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[0268] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to further purification, for example, by low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, performed at low salt concentrations (e.g., from about 0-0.25M salt).

[0269] As noted above, in some embodiments, one of the two arms of the bispecific antibody comprises a heavy chain that comprises H435R and Y436F substitutions (numbering according to EU index). When introduced into one of the two arms of a bispecific antibody, these substitutions can allow for easier purification of the correctly assembled, heterodimeric bispecific antibody over homodimeric forms using protein A affinity chromatography; *see, e.g.*, Tustian, A. *et al.* (2016) *MAbs* 8(4):828-838.

[0270] In general, various methodologies for preparing antibodies for use in research, testing, and clinical use are well-established in the art, consistent with the above-described methodologies and/or as deemed appropriate by one skilled in the art for a particular antibody of interest.

Production of non-fucosylated antibodies

[0271] Provided herein are methods for preparing bispecific antibodies with a reduced degree of fucosylation. For example, methods contemplated herein include, but are not limited to, use of cell lines deficient in protein fucosylation (e.g., Lec13 CHO cells, alpha-1,6-fucosyltransferase gene knockout CHO cells, cells overexpressing β 1,4-N-acetylglycosaminyltransferase III and further overexpressing Golgi μ -mannosidase II, etc.), and addition of a fucose analog(s) in a cell culture medium used for the production of the antibodies. See Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; WO 2004/056312 A1; Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); and US Pat. No. 8,574,907. Additional techniques for reducing the fucose content of antibodies include Glymaxx technology described in U.S. Patent Application Publication No. 2012/0214975. Additional techniques for reducing the fucose content of antibodies also include the addition of one or more glycosidase inhibitors in a cell culture medium used for the production of the antibodies. Glycosidase inhibitors include α -glucosidase I, α -glucosidase II, and α -mannosidase I. In some embodiments, the glycosidase inhibitor is an inhibitor of α -mannosidase I (e.g., kifunensine).

[0272] As used herein, “core fucosylation” refers to addition of fucose (“fucosylation”) to N-acetylglucosamine (“GlcNAc”) at the reducing terminal of an N-linked glycan. Also provided are antibodies produced by such methods and compositions thereof.

[0273] In some embodiments, fucosylation of complex N-glycoside-linked sugar chains bound to the Fc region (or domain) is reduced. As used herein, a “complex N-glycoside-linked sugar chain” is typically bound to asparagine 297 (according to the number of Kabat), although a complex N-glycoside linked sugar chain can also be linked to other asparagine residues. A “complex N-glycoside-linked sugar chain” excludes a high mannose type of sugar chain, in which only mannose is incorporated at the non-reducing terminal of the core structure, but includes 1) a complex type, in which the non-reducing terminal side of the core structure has one or more branches of galactose-N-acetylglucosamine (also referred to as “gal-GlcNAc”) and the non-reducing terminal side of Gal-GlcNAc optionally has a sialic acid, bisecting N-acetylglucosamine or the like; or 2) a hybrid type, in which the non-reducing terminal side of the core structure has both branches of the high mannose N-glycoside-linked sugar chain and complex N-glycoside-linked sugar chain.

[0274] In some embodiments, the “complex N-glycoside-linked sugar chain” includes a complex type in which the non-reducing terminal side of the core structure has zero, one or more branches of galactose-N-acetylglucosamine (also referred to as “gal-GlcNAc”) and the non-reducing terminal side of Gal-GlcNAc optionally further has a structure such as a sialic acid, bisecting N-acetylglucosamine or the like.

[0275] According to the present methods, typically only a minor amount of fucose is incorporated into the complex N-glycoside-linked sugar chain(s). For example, in various embodiments, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 1% of the antibody has core fucosylation by fucose in a composition. In some embodiments, substantially none (i.e., less than about 0.5%) of the antibody has core fucosylation by fucose in a composition. In some embodiments, more than about 40%, more than about 50%, more than about 60%, more than about 70%, more than about 80%, more than about 90%, more than about 91%, more than about 92%, more than about 93%, more than about 94%, more than about 95%, more than about 96%, more than about 97%, more than about 98%, or more than about 99% of the antibody is nonfucosylated in a composition.

[0276] In some embodiments, provided herein is a bispecific antibody wherein substantially none (i.e., less than about 0.5%) of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some embodiments, provided herein is a bispecific antibody wherein at least one or two of the heavy chains of the antibody is/are non-fucosylated.

[0277] As described above, a variety of mammalian host-expression vector systems can be utilized to express an antibody. In some embodiments, the culture media is not supplemented with fucose. In some embodiments, an effective amount of a fucose analog is added to the culture media. In this context, an “effective amount” refers to an amount of the analog that is sufficient to decrease fucose incorporation into a complex N-glycoside-linked sugar chain of an antibody by at least about 10%, at least about 20%, at least about 30%, at least about 40% or at least about 50%. In some embodiments, antibodies produced by the instant methods comprise at least about 10%, at least about 20%, at least about 30%, at least about 40% or at least about 50% non-core fucosylated protein (e.g., lacking core fucosylation), as compared with antibodies produced from the host cells cultured in the absence of a fucose analog.

[0278] The content (e.g., the ratio) of sugar chains in which fucose is not bound to N-acetylglucosamine in the reducing end of the sugar chain versus sugar chains in which fucose is bound to N-acetylglucosamine in the reducing end of the sugar chain can be determined, for example, as described in the Examples. Other methods include hydrazinolysis or enzyme digestion (see, e.g., Biochemical Experimentation Methods 23: Method for Studying Glycoprotein Sugar Chain (Japan Scientific Societies Press), edited by Reiko Takahashi (1989)), fluorescence labeling or radioisotope labeling of the released sugar chain and then separating the labeled sugar chain by chromatography. Also, the compositions of the released sugar chains can be determined by analyzing the chains by the HPAEC-PAD method (see, e.g., J. Liq Chromatogr. 6:1557 (1983)). (See generally U.S. Patent Application Publication No. 2004/0110282.).

III. Methods

[0279] Certain aspects of the present disclosure relate to methods for using the bispecific antibodies disclosed herein (e.g., *supra*). In some embodiments, the methods comprise administering to a subject or individual (e.g., in need thereof) an effective amount of a bispecific antibody of the present disclosure or a composition (e.g., a pharmaceutical composition) of the present disclosure. In some embodiments, the individual is a human.

[0280] In some embodiments, provided herein is a method for treating a disease or condition characterized by increased activity and/or number of mast cells expressing Siglec-6 and/or Siglec-8, comprising administering to a subject or individual (e.g., in need thereof) an effective

amount of a bispecific antibody of the present disclosure or a composition (*e.g.*, a pharmaceutical composition) of the present disclosure.

[0281] In some embodiments, provided herein is a method for inhibiting activation of mast cells expressing Siglec-6 and/or Siglec-8 in a subject in need thereof, comprising administering to a subject or individual an effective amount of a bispecific antibody of the present disclosure or a composition (*e.g.*, a pharmaceutical composition) of the present disclosure.

[0282] In some embodiments, provided herein is a method for depleting mast cells expressing Siglec-6 and/or Siglec-8 in a subject in need thereof, comprising administering to a subject or individual an effective amount of a bispecific antibody of the present disclosure or a composition (*e.g.*, a pharmaceutical composition) of the present disclosure.

[0283] In some embodiments, provided herein is a method for treating a disease or condition mediated by cells expressing Siglec-6 and/or Siglec-8 in a subject, comprising administering to a subject or individual (*e.g.*, in need thereof) an effective amount of a bispecific antibody of the present disclosure or a composition (*e.g.*, a pharmaceutical composition) of the present disclosure.

[0284] A variety of diseases/disorders are contemplated for treatment using the methods of the present disclosure. Exemplary diseases/disorders may be found, *e.g.*, in U.S. Pat. No. 9,546,215 and/or International Publication Nos. WO2015089117, WO2022087610, and WO2023044390. In some embodiments, the disease or disorder is mast cell-mediated. In some embodiments, the disease or disorder is eosinophil-mediated.

[0285] In some embodiments, the subject or individual has or has been diagnosed with mastocytosis, mast cell leukemia, mast cell activation syndrome, gastroparesis, osteoporosis, osteopenia, renal osteodystrophy, bone fracture, Alzheimer's disease, chronic neuropathic pain, hyperalgesia, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), graft vs. host disease (GVH), colitis, hereditary alpha tryptasemia, neurofibroma, Kounis syndrome, urticaria, atopic dermatitis, contact dermatitis, angioedema, prurigo nodularis, cholangitis, psoriasis, irritable bowel syndrome (IBS), functional dyspepsia, asthma, allergy, keloid, chronic rhinosinusitis, aspirin exacerbated respiratory disease (AERD), chronic obstructive pulmonary disease (COPD), bullous pemphigoid, idiopathic pulmonary fibrosis, systemic sclerosis, interstitial cystitis, hidradenitis suppurativa, alopecia areata, vitiligo, mast cell gastrointestinal disease, Crohn's disease, rheumatoid arthritis, gastroesophageal reflux disease, viral infection, achalasia, postural tachycardia syndrome, amyotrophic lateral sclerosis

(ALS), multiple sclerosis (MS), complex regional pain syndrome, osteoarthritis, or Ehlers-Danlos syndrome. In some embodiments, the subject or individual has or has been diagnosed with allergic rhinitis, nasal polyposis, pauci granulocytic asthma, acute or chronic airway hypersensitivity, eosinophilic esophagitis, eosinophilic leukemia, Churg-Strauss syndrome, inflammation associated with a cytokine, inflammation associated with cells expressing Siglec-8, malignancy associated with cells expressing Siglec-8, physical urticaria, cold urticaria, pressure-urticaria, bullous pemphigoid, food allergy, chronic rhinosinusitis with concomitant asthma, aspirin-exacerbated respiratory disease, adult onset non-atopic asthma with sinus disease, chronic obstructive pulmonary disease, fibrotic disease, pre-fibrotic disease, mastocytosis, advanced systemic mastocytosis, indolent systemic mastocytosis (ISM), inflammatory bowel disease (IBD), eosinophilic esophagitis (EOE), eosinophilic gastritis (EG), eosinophilic gastroenteritis (EGE), eosinophilic colitis (EOC), eosinophilic duodenitis, mast cell gastritis or mast cell gastroenteritis, gastritis or gastroenteritis with elevated mast cells, irritable bowel syndrome, irritable bowel syndrome with elevated mast cells, functional gastrointestinal disease, functional dyspepsia, allergic conjunctivitis, giant papillary conjunctivitis, chronic urticaria, allergic bronchopulmonary aspergillosis (ABPA), allergic asthma, asthma with eosinophil or mast cell phenotype, eosinophilic granulomatosis with polyangiitis (EGPA), celiac disease, gastroparesis, hypereosinophilic syndrome, atopic dermatitis, anaphylaxis, angioedema, mast cell activation syndrome/disorder, or eosinophilic fasciitis.

[0286] In some embodiments, the disease or disorder is mastocytosis, mast cell leukemia, mast cell activation syndrome, gastroparesis, osteoporosis, osteopenia, renal osteodystrophy, bone fracture, Alzheimer's disease, chronic neuropathic pain, hyperalgesia, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), graft vs. host disease (GVH), colitis, hereditary alpha tryptasemia, neurofibroma, Kounis syndrome, urticaria, atopic dermatitis, contact dermatitis, angioedema, prurigo nodularis, cholangitis, psoriasis, irritable bowel syndrome (IBS), functional dyspepsia, asthma, allergy, keloid, chronic rhinosinusitis, aspirin exacerbated respiratory disease (AERD), chronic obstructive pulmonary disease (COPD), bullous pemphigoid, idiopathic pulmonary fibrosis, systemic sclerosis, interstitial cystitis, hidradenitis suppurativa, alopecia areata, vitiligo, mast cell gastrointestinal disease, Crohn's disease, rheumatoid arthritis, gastroesophageal reflux disease, viral infection, achalasia, postural tachycardia syndrome, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), complex regional pain syndrome, osteoarthritis, or Ehlers-Danlos syndrome. In some embodiments, the

disease or disorder is allergic rhinitis, nasal polyposis, pauci granulocytic asthma, acute or chronic airway hypersensitivity, eosinophilic esophagitis, eosinophilic leukemia, Churg-Strauss syndrome, inflammation associated with a cytokine, inflammation associated with cells expressing Siglec-8, malignancy associated with cells expressing Siglec-8, physical urticaria, cold urticaria, pressure-urticaria, bullous pemphigoid, food allergy, chronic rhinosinusitis with concomitant asthma, aspirin-exacerbated respiratory disease, adult onset non-atopic asthma with sinus disease, chronic obstructive pulmonary disease, fibrotic disease, pre-fibrotic disease, mastocytosis, advanced systemic mastocytosis, indolent systemic mastocytosis (ISM), inflammatory bowel disease (IBD), eosinophilic esophagitis (EOE), eosinophilic gastritis (EG), eosinophilic gastroenteritis (EGE), eosinophilic colitis (EOC), eosinophilic duodenitis, mast cell gastritis or mast cell gastroenteritis, gastritis or gastroenteritis with elevated mast cells, irritable bowel syndrome, irritable bowel syndrome with elevated mast cells, functional gastrointestinal disease, functional dyspepsia, allergic conjunctivitis, giant papillary conjunctivitis, chronic urticaria, allergic bronchopulmonary aspergillosis (ABPA), allergic asthma, asthma with eosinophil or mast cell phenotype, eosinophilic granulomatosis with polyangiitis (EGPA), celiac disease, gastroparesis, hypereosinophilic syndrome, atopic dermatitis, anaphylaxis, angioedema, mast cell activation syndrome/disorder, or eosinophilic fasciitis.

[0287] In some embodiments, administration of a composition or bispecific antibody of the present disclosure results in a decrease in one or more symptoms in the subject, *e.g.*, as compared to a reference, reference value, or the one or more symptoms in the subject prior to the administration. Exemplary symptoms include, without limitation, nausea, cramping, constipation, abdominal pain, bloating, vomiting, diarrhea, fatigue, eye pain, light sensitivity, redness, discharge, runny nose, headache, dizziness, brain fog, itching, flushing, sweating, hives, hypotension, shortness of breath, bone pain, joint pain, weight loss, osteoporosis, angioedema, chest pain, anxiety, depression, rapid heartbeat, bronchoconstriction, and general pain.

[0288] In some embodiments, administration of a composition or bispecific antibody of the present disclosure results in a sustained response to treatment. In some embodiments, administration of a composition or antibody of the present disclosure results in a complete response to treatment (*e.g.*, after cessation of treatment, or after a single dose of the antibody or composition).

[0289] The terms “reference” or “reference value” used interchangeably herein can refer to a measurement or characterization of a value or symptom in an individual. A “reference value”

can be an absolute value; a relative value; a value that has an upper and/or lower limit; a range of values; an average value; a median value; a mean value; or a value as compared to a baseline value. Similarly, a “baseline value” can be an absolute value; a relative value; a value that has an upper and/or lower limit; a range of values; an average value; a median value; a mean value; or a value as compared to a reference value. A reference value can be obtained from one individual, from two different individuals or from a group of individuals (*e.g.*, a group of two, three, four, five or more individuals). In some embodiments, a reference value refers to a standard or benchmark value in the field. In some embodiments, a reference value refers to a value calculated *de novo* from one or more individuals.

[0290] For the prevention or treatment of disease, the appropriate dosage of an active agent, will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the individual's clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to the individual at one time or over a series of treatments. Any dosing frequency described above may be used in the methods or uses of the compositions described herein. Efficacy of treatment with an antibody described herein can be assessed using any of the methodologies or assays described herein at intervals ranging between every week and every three months. In some embodiments, efficacy of treatment (*e.g.*, reduction or improvement of one or more symptoms) is assessed about every one month, about every two months, about every three months, about every four months, about every five months, about every six months or longer after administration of an antibody. In some embodiments, efficacy of treatment (*e.g.*, reduction or improvement of one or more symptoms) is assessed about every one week, about every two weeks, about every three weeks, about every four weeks, about every five weeks, about every six weeks, about every seven weeks, about every eight weeks, about every nine weeks, about every ten weeks, about every eleven weeks, about every twelve weeks, about every sixteen weeks, about every twenty weeks, about every twenty four weeks, or longer.

[0291] Bispecific antibodies described herein can be used either alone or in combination with other agents in the methods described herein. For instance, a bispecific antibody may be co-administered with one or more (*e.g.*, one or more, two or more, three or more, four or more, *etc.*) additional therapeutic agents.

[0292] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the present disclosure can occur prior to, simultaneously, and/or following, administration of the one or more additional therapeutic agents.

[0293] Bispecific antibodies and/or one or more additional therapeutic agents may be administered via any suitable route of administration known in the art, including, without limitation, by oral administration, sublingual administration, buccal administration, topical administration, rectal administration, via inhalation, transdermal administration, subcutaneous injection, intradermal injection, intravenous (IV) injection, intra-arterial injection, intramuscular injection, intracardiac injection, intraosseous injection, intraperitoneal injection, transmucosal administration, vaginal administration, intravitreal administration, intra-articular administration, peri-articular administration, local administration, epicutaneous administration, or any combinations thereof. In some embodiments, the bispecific antibody or composition of the present disclosure is administered by subcutaneous injection. In some embodiments, the bispecific antibody or composition of the present disclosure is administered by intravenous infusion.

IV. Compositions

[0294] In some aspects, also provided herein are compositions (e.g., pharmaceutical compositions) comprising any of the bispecific antibodies described herein. In some aspects, provided herein is a composition comprising a bispecific antibody described herein, wherein the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than about 50% of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some embodiments, the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, or about 15% of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some aspects, provided herein is a composition comprising a bispecific antibody described herein, wherein the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue.

[0295] Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington: The Science and Practice of Pharmacy, 20th Ed., Lippincott Williams & Wilkins, Pub., Gennaro Ed., Philadelphia, Pa. 2000). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite; preservatives, isotonicifiers, stabilizers, metal complexes (e.g., Zn-protein complexes); chelating agents such as EDTA and/or non-ionic surfactants.

[0296] Buffers can be used to control the pH in a range which optimizes the therapeutic effectiveness, especially if stability is pH dependent. Buffers can be present at concentrations ranging from about 50 mM to about 250 mM. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof. For example, citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate. Additionally, buffers may be comprised of histidine and trimethylamine salts such as Tris.

[0297] Preservatives can be added to prevent microbial growth, and are typically present in a range from about 0.2%-1.0% (w/v). Suitable preservatives for use with the present disclosure include octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium halides (e.g., chloride, bromide, iodide), benzethonium chloride; thimerosal, phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol, 3-pentanol, and m-cresol.

[0298] Tonicity agents, sometimes known as “stabilizers” can be present to adjust or maintain the tonicity of liquid in a composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed “stabilizers” because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter and intra-molecular interactions. Tonicity agents can be present in any amount between about 0.1% to about 25% by weight or between about 1 to about 5% by weight, taking into account the relative amounts of the other ingredients. In some embodiments, tonicity agents include polyhydric sugar alcohols, trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[0299] Additional excipients include agents which can serve as one or more of the following: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) agents preventing denaturation or adherence to the container wall. Such excipients include: polyhydric sugar

alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinositol, myoinositol, galactose, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (e.g., xylose, mannose, fructose, glucose; disaccharides (e.g., lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

[0300] Non-ionic surfactants or detergents (also known as “wetting agents”) can be present to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Non-ionic surfactants are present in a range of about 0.05 mg/ml to about 1.0 mg/ml or about 0.07 mg/ml to about 0.2 mg/ml. In some embodiments, non-ionic surfactants are present in a range of about 0.001% to about 0.1% w/v or about 0.01% to about 0.1% w/v or about 0.01% to about 0.025% w/v.

[0301] Suitable non-ionic surfactants include polysorbates (20, 40, 60, 65, 80, etc.), polyoxamers (184, 188, etc.), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.), laurmacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride.

[0302] In order for the formulations to be used for in vivo administration, they must be sterile. The formulation may be rendered sterile by filtration through sterile filtration membranes. The therapeutic compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0303] The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, e.g.,

injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intraarterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means.

[0304] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active compounds are suitably present in combination in amounts that are effective for the purpose intended.

V. Articles of Manufacture or Kits

[0305] In another aspect, an article of manufacture or kit is provided which comprises a bispecific antibody described herein. The article of manufacture or kit may further comprise instructions for use of the antibody in the methods of the present disclosure. Thus, in certain embodiments, the article of manufacture or kit comprises instructions for the use of a bispecific antibody that binds to human Siglec-8 and human Siglec-6 in methods of the present disclosure. In certain embodiments, the article of manufacture comprises a medicament comprising a bispecific antibody of the present disclosure and a package insert comprising instructions for administration of the medicament in an individual in need thereof according to any of the methods disclosed herein. In some embodiments, the package insert further indicates that the treatment is effective in reducing one or more symptoms in the individual as compared to a baseline level before administration of the medicament. In certain embodiments, the individual is a human.

[0306] The article of manufacture or kit may further comprise a container. Suitable containers include, for example, bottles, vials (e.g., dual chamber vials), syringes (such as single or dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation.

[0307] The article of manufacture or kit may further comprise a label or a package insert, which is on or associated with the container, may indicate directions for reconstitution and/or use of the formulation. The label or package insert may further indicate that the formulation is useful or intended for subcutaneous, intravenous, or other modes of administration. The container holding the formulation may be a single-use vial or a multi-use vial, which allows for repeat administrations of the reconstituted formulation. The article of manufacture or kit may further comprise a second container comprising a suitable diluent. The article of manufacture or

kit may further include other materials desirable from a commercial, therapeutic, and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0308] In a specific embodiment, the present disclosure provides kits for a single dose-administration unit. Such kits comprise a container of an aqueous formulation of therapeutic antibody, including both single or multi-chambered pre-filled syringes. Exemplary pre-filled syringes are available from Vetter GmbH, Ravensburg, Germany.

[0309] In another embodiment, provided herein is an article of manufacture or kit comprising the formulations described herein for administration in an auto-injector device. An auto-injector can be described as an injection device that upon activation, will deliver its contents without additional necessary action from the patient or administrator. They are particularly suited for self-medication of therapeutic formulations when the delivery rate must be constant and the time of delivery is greater than a few moments.

[0310] It is understood that the aspects and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

EXAMPLES

[0311] The present disclosure will be more fully understood by reference to the following examples. The examples should not, however, be construed as limiting the scope of the present disclosure. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: Bispecific Siglec-6/8 antibody induces internalization of both Siglec-6 and Siglec-8

Materials and Methods

[0312] Fresh human lung tissue was procured and provided by the National Cancer Institute (NCI) Cooperative Human Tissue Network (CHTN) from subjects with no previous history of chronic disease. Human tissues were enzymatically and mechanically dissociated using the

gentleMACs™ Dissociator (Miltenyi Biotec), according to manufacturer's protocol. After the last digestion, the tissue was filtered through a 70um filter and red blood cells were lysed using RBC lysis buffer (Biolegend). Single cells were then washed and resuspended into RPMI media with 10% super low IgG FBS and P/S. Immediately after digestion, cell viability was examined using flow cytometry. From dissociated tissues, only single-cell suspensions that had at least 70% viability were used in subsequent experiments.

[0313] For flow cytometry, cells were preincubated with Human or Mouse Fc Block (BD Biosciences) to block nonspecific binding. Cells were then incubated at 4°C for 10 min with staining antibody panels, washed, and fixed in 2% paraformaldehyde. The marker CD45 was used to identify hematopoietic cells, and 7AAD was used as a viability dye. Human mast cells were identified as CD45+ 7AAD- HLA-DR- CD11b- CD117+ FcεRI+. Mouse mast cells were identified as CD45+ 7AAD- CD117+ FcεRI+. Mouse peripheral blood eosinophils were identified as CD45+ 7AAD- CD11b+ Ly6C- Ly6G- SiglecF+. Data acquisition was performed using a NovoCyte flow cytometer and FlowJo was used for data analysis.

Results

[0314] Human lung mast cells were cultured with varying concentrations of the bispecific Siglec-6/8 huIgG1, anti-Siglec-6 huIgG1 (comprising the VH and VL regions of SEQ ID NO:254 and 255, respectively), anti-Siglec-8 afucosylated huIgG1 (comprising the VH and VL regions of SEQ ID NO:6 and 16, respectively), or an isotype control for 18h at 37°C. Internalization was measured by FACS using a fluorescent-tagged Siglec-6 or Siglec-8 mAb that binds to a different epitope on Siglec-6 or Siglec-8. Median fluorescence intensity (MFI) values were corrected by subtracting the MFI of a Fluorescence-Minus-One (FMO) control to generate delta MFI (dMFI) values. Both the fucosylated and afucosylated forms of the bispecific Siglec-6/8 huIgG1 induced dose-dependent internalization of Siglec-6 (**FIG. 2A**) and Siglec-8 (**FIG. 2B**) on human mast cells. Anti-Siglec-8 afucosylated huIgG1 induced internalization of Siglec-8 only (**FIG. 2B**), while anti-Siglec-6 huIgG1 induced internalization of Siglec-6 only (**FIG. 2A**).

Example 2: Bispecific Siglec-6/8 antibody inhibits human mast cell activation

[0315] Human lung mast cells were stimulated with an anti-FcεRI (10μg/mL) in the presence of the bispecific Siglec-6/8 huIgG1, anti-Siglec-6 huIgG1, anti-Siglec-8 huIgG1, or an isotype control at indicated concentrations. Bound antibodies were crosslinked with 20 μg/mL

secondary antibody AffiniPure F(ab')₂ Fragment Goat anti-human IgG heavy chain and light chain (H+L). Cells were incubated for 20 min at 37°C. Activation was assessed by flow cytometry using the activation marker CD63. The bispecific Siglec-6/8 huIgG1 and anti-Siglec-6 huIgG1 showed similar inhibitory activity on mast cells, while anti-Siglec-8 huIgG1 was less potent at inhibiting mast cell activation (**FIG. 3**).

Example 3: Bispecific Siglec-6/8 antibody induces Siglec-8 receptor internalization and depletes eosinophils

[0316] Siglec-6/ Siglec-8 double transgenic mice were dosed intraperitoneally with 5mg/kg of bispecific Siglec-6/8 huIgG1, bispecific Siglec-6/8 afucosylated huIgG1, anti-Siglec-6 huIgG1, anti-Siglec-8 afucosylated huIgG1, or an isotype control for 24h. Blood was processed through two rounds of red blood cell lysis, according to manufacturer instructions (Biolegend). The cell pellet was resuspended in RPMI complete media. Internalization was measured by FACS using a fluorescent-tagged Siglec-8 mAb that binds to a different epitope on Siglec-8. The bispecific Siglec-6/8 huIgG1, bispecific Siglec-6/8 afucosylated huIgG1, and anti-Siglec-8 afucosylated huIgG1 antibodies induced Siglec-8 internalization on eosinophils (**FIG. 4A**) as well as eosinophil depletion (**FIG. 4B**).

[0317] To assess ADCC activity, human peripheral blood NK cells were purchased from StemCell. The day prior to the ADCC assay, the cells were thawed and cultured overnight in media containing interleukin-2 (5 ng/mL). The next day, human blood eosinophils were purified using a human eosinophil isolation kit from StemCell. Eosinophils were plated in the presence of NK cells at a ratio of 9:1 NK:eosinophils and cultured for 18h at 37°C with specified concentrations of the bispecific Siglec-6/8 huIgG1, bispecific Siglec 6/8 afucosylated huIgG1, anti-Siglec-6 huIgG1, anti-Siglec-8 afucosylated huIgG1, or an isotype control. After incubation, cells were resuspended in AnnexinV binding buffer with AnnexinV and 7AAD. Cells were stained in the dark at room temperature for 30 min and then data were acquired by flow cytometry. Total eosinophils were identified as SSChi, and viable eosinophils were further identified as AnnexinV- 7AAD-. The bispecific Siglec-6/8 huIgG1, bispecific Siglec-6/8 afucosylated huIgG1, and anti-Siglec-8 afucosylated huIgG1 antibodies induced ADCC of human peripheral blood eosinophils (**FIG. 4C**).

Example 4: Bispecific Siglec-6/8 antibody induces internalization of both Siglec-6 and Siglec-8 on mouse tissue mast cells

[0318] Siglec-6/Siglec-8 double transgenic mice were intraperitoneally dosed with 5mg/kg of bispecific Siglec-6/8 huIgG1, bispecific Siglec-6/8 afucosylated huIgG1, anti-Siglec-6 huIgG1, anti-Siglec-8 afucosylated huIgG1, or an isotype control for 24h. The stomachs were collected, and the intraepithelial layer was released by incubation in Hank's Balanced Salt Solution (HBSS) without magnesium or calcium (Gibco), with 5 mM EDTA and 5% FBS and passed through a 70um filter. Internalization was measured by FACS using a fluorescent-tagged Siglec-6 or Siglec-8 mAb that binds to a different epitope on Siglec-6 or Siglec-8. Both the bispecific Siglec-6/8 huIgG1 and bispecific Siglec-6/8 afucosylated huIgG1 induced internalization of Siglec-6 (**FIG. 5B**) and Siglec-8 (**FIG. 5A**) on tissue mast cells. The anti-Siglec-8 afucosylated huIgG1 induced internalization of Siglec-8 only, while anti-Siglec-6 huIgG1 induced internalization of Siglec-6 only (**FIG. 5A & 5B**).

Example 5: Bispecific Siglec-6/8 antibody inhibits tissue mast cell activation in vivo and ex vivo

[0319] Siglec-6/Siglec-8 double transgenic mice were intraperitoneally dosed with 5mg/kg of bispecific Siglec-6/8 huIgG1, bispecific Siglec-6/8 afucosylated huIgG1, anti-Siglec-6 huIgG1, anti-Siglec-8 afucosylated huIgG1, or an isotype control for 24h. The stomachs were collected, and the intraepithelial layer was released by incubation in Hank's Balanced Salt Solution (HBSS) without magnesium or calcium (Gibco), with 5 mM EDTA and 5% FBS and passed through a 70um filter. The anti-Siglec-6 huIgG1, bispecific Siglec-6/8 huIgG1, and the bispecific Siglec-6/8 afucosylated huIgG1 antibodies induced mast cell inhibition as assessed by reduced expression of the activation marker CD63 on tissue mast cells (**FIG. 6A**).

[0320] To assess *ex vivo* mast cell inhibition, Siglec-6/Siglec-8 double transgenic mice were intraperitoneally dosed with 10mg/kg of bispecific Siglec-6/8 huIgG1, bispecific Siglec-6/8 afucosylated huIgG1, anti-Siglec-6 huIgG1, anti-Siglec-8 huIgG1, or an isotype control for 7 days. Mice were sacrificed, and the peritoneal mast cells were harvested. The cells were treated with biotinylated anti-FcεRI and crosslinked with 5 µg/mL Neutravidin. Cells were incubated for 20 min at 37°C. Activation was assessed by flow cytometry using the activation marker CD63. The anti-Siglec-6 huIgG1, bispecific Siglec-6/8 huIgG1, and bispecific Siglec-6/8

afucosylated huIgG1 antibody treatments induced mast cell inhibition as assessed by reduced expression of the activation marker CD63 on tissue mast cells (**FIG. 6B**).

Example 6: Bispecific Siglec-6/8 antibody induces mast cell reduction

[0321] CD14⁺ Monocytes were isolated using the EasySep Human Monocyte Isolation kit from healthy blood donors. Monocytes were cultured in the presence of 50 ng/mL macrophage colony stimulating-factor (M-CSF) and differentiated for 6-8 days. Macrophages were harvested by treating with TrypLE solution for 5 minutes to release cells.

[0322] Human lung tissue cells were plated in the presence of IFN γ (50ng/ml)-activated monocyte-derived macrophages at a ratio of 50:1 macrophages:mast cells and cultured for 18h at 37C with specified concentrations of bispecific Siglec-6/8 huIgG1, bispecific Siglec-6/8 afucosylated huIgG1, anti-Siglec-6 huIgG1, anti-Siglec-8 afucosylated huIgG1, or an isotype control. ADCP was measured by the loss of the mast cell population. The anti-Siglec-6 huIgG1, bispecific Siglec-6/8 huIgG1, and bispecific Siglec-6/8 afucosylated huIgG1 antibody treatments induced depletion of human tissue mast cells, whereas anti-Siglec-8 afucosylated huIgG1 treatment displayed only mild mast cell depletion (**FIG. 7**).

[0323] These results indicate that bispecific Siglec-6/8 huIgG1 and bispecific Siglec-6/8 afucosylated huIgG1 antibodies induce ADCC of eosinophils, internalization of Siglec-6 and Siglec-8, mast cell inhibition, and mast cell reduction.

Example 7: Functional bispecific Siglec-6/8 antibodies can be generated in various formats

[0324] Different formats for bispecific Siglec-6/8 antibodies were tested for the ability to bind both Siglec-6 and Siglec-8, as well as different configurations for which arm bound to Siglec-6 and which bound to Siglec-8. Activity was assessed by evaluating binding to Siglec-6 and Siglec-8 using ELISA. As shown in Table 9, all bispecific antibodies generated bound to Siglec-6 and Siglec-8.

Table 9. Bispecific antibody formats tested.

Design	Binding to Siglec-6	Binding to Siglec-8
DuetMab + KIH	+	+
CrossMab (Siglec-8 ^{CH1}) + KIH	+	+
CrossMab (Siglec-6 ^{CH1}) + KIH	+	+

CrossMab (Siglec-8 ^{VH}) + KIH	+	+
CrossMab (Siglec-6 ^{VH}) + KIH	+	+

KIH, knobs-into-holes.

Example 8: Global protein interactions of Siglec-6 and Siglec-8 reveal distinct differences in regulating mast cell function

[0325] Sialic acid-binding immunoglobulin-like lectin (Siglec)-6 and Siglec-8 are receptors with broad mast cell (MC) inhibitory activity and therefore potential therapeutic targets in diseases driven by MCs. The functional differences between these closely related receptors are incompletely understood. Here, unbiased proteomic profiling was used to identify membrane and intracellular proteins that interact with Siglec-6 or Siglec-8 (i.e., ‘interactome’) in MCs.

[0326] Components of Siglec-6 and Siglec-8 protein complexes immuno-precipitated from primary MCs were identified by mass-spectrometry (**FIG. 8A**). Detailed interaction with KIT/CD117 and inhibition of stem cell factor (SCF)-mediated MC activation was evaluated with anti-Siglec-6 and Siglec-8 antibodies.

[0327] Siglec-6 and Siglec-8 interactomes were comprised of multiple critical MC activating receptors and signaling molecules, including KIT, the high affinity receptor for IgE, (subunits of) receptors for IL-3, IL-4, and IL-33, the intracellular kinases LYN and JAK1, and the phosphatases CD45, SHP-1 and SHP-2. Based on protein interaction networks and pathway analysis, Siglec-6 appeared to have a more substantive regulatory role than Siglec-8. Siglec-6 specifically interacted with multiple subunits of the FcεRI receptor, proteins involved in microtubule dynamics and degranulation, and multiple proteins involved in MC metabolism. The pathways enriched in the Siglec-6, but not Siglec-8 interactome, included SCF/KIT, thymic stromal lymphopoietin signaling, pyruvate dehydrogenase activity, and regulation of metabolic processes (**FIG. 8B**). Notably, Siglec-6, but not Siglec-8 interacted with the mature, glycosylated form of KIT which resulted in inhibition of SCF-mediated MC activation with an agonistic anti-Siglec-6 antibody.

[0328] These data demonstrate distinct roles for Siglec-6 and Siglec-8 in controlling MC activation through interaction with multiple activating receptors and key signaling molecules.

SELECTED SEQUENCES

All polypeptide sequences are presented N-terminal to C-terminal unless otherwise noted.

All nucleic acid sequences are presented 5' to 3' unless otherwise noted.

Amino acid sequence of mouse 2E2 heavy chain variable domain

QVQLKESGPGLVAPSQSLITCTVSGFSLTIYGAHWVRQPPGKGLEWLGVIWAGGSTNY
NSALMSRLSISKDNSKSQVFLKINSLQTDDTALYYCARDGSSPYYYSM EYWGGQTSVT
VSS (SEQ ID NO:1)

Amino acid sequence of 2E2 RHA heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVSVIWAGGSTN
YNSALMSRFTISKDNSKNTVY LQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGGQGT
VTVSS (SEQ ID NO:2)

Amino acid sequence of 2E2 RHB heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAVSGFSLTIYGAHWVRQAPGKGLEWLGVIWAGGSTN
YNSALMSRLSISKDNSKNTVY LQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGGQGT
VTVSS (SEQ ID NO:3)

Amino acid sequence of 2E2 RHC heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAVSGFSLTIYGAHWVRQAPGKGLEWVSVIWAGGSTN
YNSALMSRFTISKDNSKNTVY LQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGGQGT
VTVSS (SEQ ID NO:4)

Amino acid sequence of 2E2 RHD heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWLSVIWAGGSTN
YNSALMSRFTISKDNSKNTVY LQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGGQGT
VTVSS (SEQ ID NO:5)

Amino acid sequence of 2E2 RHE heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYNSALMSRFTISKDNSKNTVY LQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGGQGT
TVTSS (SEQ ID NO:6)

Amino acid sequence of 2E2 RHF heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVSVIWAGGSTN
YNSALMSRLTISKDNSKNTVY LQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGGQGT
VTVSS (SEQ ID NO:7)

Amino acid sequence of 2E2 RHG heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVSVIWAGGSTN
YNSALMSRFSISKDNSKNTVY LQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGGQGT
VTVSS (SEQ ID NO:8)

Amino acid sequence of 2E2 RHA2 heavy chain variable domain

QVQLQESGPGLVKPSETLSLTCTVSGGSISIYGAHWIRQPPGKGLEWIGVIWAGGSTNYN
SALMSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDGSSPYYYSM EYWGQGT LVTV
SS (SEQ ID NO:9)

Amino acid sequence of 2E2 RHB2 heavy chain variable domain

QVQLQESGPGLVKPSETLSLTCTVSGFSLTIYGAHWVRQPPGKGLEWLGVWAGGSTN
YNSALMSRLSISKDNSKNQVSLKLSSVTAADTAVYYCARDGSSPYYYSM EYWGQGT L
VTVSS (SEQ ID NO:10)

Amino acid sequence of 2E2 RHE S-G mutant heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYGMEYWGQGT
TVTSS (SEQ ID NO:11)

Amino acid sequence of 2E2 RHE E-D heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSM DYWGQGT L
TVTSS (SEQ ID NO:12)

Amino acid sequence of 2E2 RHE Y-V heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSM EVWGQGT L
TVTSS (SEQ ID NO:13)

Amino acid sequence of 2E2 RHE triple mutant heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYGMDVWGQG
TTVTSS (SEQ ID NO:14)

Amino acid sequence of mouse 2E2 light chain variable domain

QIILTQSPAIMSASPGEKVSITCSATSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVVPRF
SGSGSGTSSYSLTISRMEAEDAATYYCQQRSSYPFTFGSGTKLEIK (SEQ ID NO:15)

Amino acid sequence of 2E2 RKA light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:16)

Amino acid sequence of 2E2 RKB light chain variable domain

EIILTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLWIYSTSNLASGVPARF
SGSGSGTDYTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:17)

Amino acid sequence of 2E2 RKC light chain variable domain

EIILTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARFS
GSGSGTDFTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:18)

Amino acid sequence of 2E2 RKD light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLWIYSTSNLASGIPARF
SGSGSGTDFTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:19)

Amino acid sequence of 2E2 RKE light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGVPAR
FSGSGSGTDFTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:20)

Amino acid sequence of 2E2 RKF light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDYTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:21)

Amino acid sequence of 2E2 RKG light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:22)

Amino acid sequence of 2E2 RKA F-Y mutant light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSELPEDFAVYYCQQRSSYPYTFGPGTKLDIK (SEQ ID NO:23)

Amino acid sequence of 2E2 RKF F-Y mutant light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDYTLTISSELPEDFAVYYCQQRSSYPYTFGPGTKLDIK (SEQ ID NO:24)

Amino acid sequence of HEKA heavy chain and HEKF heavy chain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYSMEYWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFLL
YSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPG (SEQ ID NO:75)

Amino acid sequence of HEKA light chain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:76)

Amino acid sequence of HEKF light chain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDYTLTISSELPEDFAVYYCQQRSSYPFTFGPGTKLDIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:77)

Amino acid sequence of IgG1 heavy chain constant region

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:78)

Amino acid sequence of IgG4 heavy chain constant region

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFCFSVMHEALHNHYTQKSLSLSLG (SEQ ID NO:79)

Amino acid sequence of Ig kappa light chain constant region

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:80)

Amino acid sequence of murine 2C4 and 2E2 IgG1 heavy chain

QVQLKRA SGPLVAPSQSL SITCTVSGFSLTIYGAHWVRQPPGKGLEWLGVIWAGGSTN
YNSALMSRLSISKDNSKSQVFLKINSLQTDDTALYYCARDGSSPYYYSM EYWGGTSV
TVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPA
VLESDLYTLSSSVTVPSSPRPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSS
VFIFPPKPKDVLITLTPKVTCTVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNST
FRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMA
KDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMNTNGSYFVYSKLVNQKSN
WEAGNTFTCSVLHEGLHNHHT EKSLSHSPG (SEQ ID NO:81)

Amino acid sequence of murine 2C4 kappa light chain

EIILTQSPA IMSASPGEKVSITCSATSSVS YMHWFQQKPGTSPKLWIYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAE DAATYYCQQRSSYPFTFGSGTKLEIKADAAPT VSIFFPSSEQ
LTSGGASVVCFLNNFY PKDINVKWKIDG SERQNGVLNSWTDQDSKDSTYSMSSTLTLT
KDEYERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID NO:82)

Amino acid sequence of murine 2E2 kappa light chain

QIILTQSPA IMSASPGEKVSITCSATSSVS YMHWFQQKPGTSPKLWIYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAE DAATYYCQQRSSYPFTFGSGTKLEIKADAAPT VSIFFPSSEQ
LTSGGASVVCFLNNFY PKDINVKWKIDG SERQNGVLNSWTDQDSKDSTYSMSSTLTLT
KDEYERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID NO:83)

Amino acid sequence of chimeric 2C4 and 2E2 IgG1 heavy chain

QVQLKRA SGPLVAPSQSL SITCTVSGFSLTIYGAHWVRQPPGKGLEWLGVIWAGGSTN
YNSALMSRLSISKDNSKSQVFLKINSLQTDDTALYYCARDGSSPYYYSM EYWGGTSV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP

REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:84)

Amino acid sequence of chimeric 2C4 kappa light chain

EIILTQSPAIMASAPGEKVSITCSATSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAEDAATYYCQQRSSYPFTFGSGTKLEIKRTVAAPS VFIFPPSDE
QLKSGTASVVCLLNNFYPRKAVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLT
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:85)

Amino acid sequence of chimeric 2E2 kappa light chain

QIILTQSPAIMASAPGEKVSITCSATSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAEDAATYYCQQRSSYPFTFGSGTKLEIKRTVAAPS VFIFPPSDE
QLKSGTASVVCLLNNFYPRKAVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLT
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:86)

Amino acid sequence of HEKA IgG4 heavy chain (IgG4 contains a S228P mutation)

EVQLVESGGGLVQPGGSLRLSCAASGFSLTITYGAAHWRQAPGKGLEWVGVIWAGGST
NYSALMSRFTISKDNSKNTVYLQMNSLR AEDTAVYYCARDGSSPYYSMEYWGQGT
TVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPTVSWNSGALTSGVHTF
AVLQSSGLYSLSSVVTVPSSSLGTQYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPEF
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRL
TVDKSRWQEGNVFCFSVMHEALHNHYTQKSLSLSLG (SEQ ID NO:87)

Amino acid sequence of mouse 1C3 heavy chain variable domain (underlined residues comprise CDRs H1 and H2 according to Chothia numbering)

EVQVVESGGDLVKS GGS LKLS CAAS GFPFSSY AMSWVRQTPDKRLEWVAI SSGGSYTY
YSDSVKGRFTISRDN AKNTLYLQMSS LK SEDTAMYYCARHETAQA AWFAYWGQGT
LVSA (SEQ ID NO:106)

Amino acid sequence of mouse 1H10 heavy chain variable domain (underlined residues comprise CDRs H1 and H2 according to Chothia numbering)

EVQLQQSGAELVRPGASVKLSCTAS GFNIKDY MYWVKQRPEQGLEWIGRI APEDGDT
EYAPKFQGKATVTADTSSNTAYLHLSSLTSEDTAVYYCTTEGNYYGSSILDYWGQGT
LTVSS (SEQ ID NO:107)

Amino acid sequence of mouse 4F11 heavy chain variable domain (underlined residues comprise CDRs H1 and H2 according to Chothia numbering)

QVQLQQSGAELVKPGASVKISCKAS G YAFRSS WMNWVKQRPGKGLEWIG QIYPGDDY
TNYNGKFKGKVTLTADRSSSTAYMQLSSLTSEDSAVYFCARLGPYGP FADWGQGT
LVSA (SEQ ID NO:108)

Amino acid sequence of mouse 1C3 light chain variable domain

QIVLTQSPAIMASAPGEKVTMTCSASSVSYMHWFQQKSGTSPKRWIYDTSKLAYGVP
ARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPPTFGGGTKLEIK (SEQ ID NO:109)

Amino acid sequence of mouse 1H10 light chain variable domain

DIQMTQTTSSLASLGDRVTISCRASQDITNYLNWYQQKPDGTVKLLIYFTSRLHSGVPS
RFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPWTFGGGKLEIK (SEQ ID NO:110)

Amino acid sequence of mouse 4F11 light chain variable domain

QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMYWYQQRPGSSPRLLIYDTSSLASGVVPR
FSGSGSGTSYSLTISRIESEDAANYCQQWNSDPYTFGGGKLEIK (SEQ ID NO:111)

Amino acid sequence of human Siglec-8 Domain 1

MEGDRQYGDGYLLQVQELVTVQEGLCVHVPCSFSYPQDGWTDSDPVHGYWFRAGDR
PYQDAPVATNNPDREVQAETQGRFQLLGDIWSNDCSLSIRDARKRDKGSYFFRLERGS
MKWSYKSQLNYKTKQLSVFVTALTHRP (SEQ ID NO:112)

Amino acid sequence of human Siglec-8 Domain 2

DILILGTLESGHSRNLTCVSPWACKQGTPPMISWIGASVSSPGPTTARSSVLTLTPKPQDH
GTSLTCQVTLPGTGVTSTVRLDVS (SEQ ID NO:113)

Amino acid sequence of human Siglec-8 Domain 3

YPPWNLMTVFQGDASTALGNGSSLSVLEGQSLRLVCAVNSNPPARLSWTRGSLTL
CPSRSSNPGLLELPRVHVRDEGEFTCRAQNAQGSQHISLSLQNEGTTGTSRPVSQVTLA
AVGG (SEQ ID NO:114)

Amino acid sequence of human Siglec-8 Domain 1 Fusion Protein

MEGDRQYGDGYLLQVQELVTVQEGLCVHVPCSFSYPQDGWTDSDPVHGYWFRAGDR
PYQDAPVATNNPDREVQAETQGRFQLLGDIWSNDCSLSIRDARKRDKGSYFFRLERGS
MKWSYKSQLNYKTKQLSVFVTALTHRPIEGRSDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:115)

Amino acid sequence of human Siglec-8 Domains 1 and 2 Fusion Protein

MEGDRQYGDGYLLQVQELVTVQEGLCVHVPCSFSYPQDGWTDSDPVHGYWFRAGDR
PYQDAPVATNNPDREVQAETQGRFQLLGDIWSNDCSLSIRDARKRDKGSYFFRLERGS
MKWSYKSQLNYKTKQLSVFVTALTHRPDILILGTLESGHSRNLTCVSPWACKQGTPPMI
SWIGASVSSPGPTTARSSVLTLTPKPQDHGTSLTCQVTLPGTGVTSTVRLDVSIEGRSD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID
NO:116)

Amino acid sequence of human Siglec-8 Domains 1, 2, and 3 Fusion Protein

MEGDRQYGDGYLLQVQELVTVQEGLCVHVPCSFSYPQDGWTDSDPVHGYWFRAGDR
PYQDAPVATNNPDREVQAETQGRFQLLGDIWSNDCSLSIRDARKRDKGSYFFRLERGS
MKWSYKSQLNYKTKQLSVFVTALTHRPDILILGTLESGHSRNLTCVSPWACKQGTPPMI
SWIGASVSSPGPTTARSSVLTLTPKPQDHGTSLTCQVTLPGTGVTSTVRLDVSYPWN

LTMTVFQGDASTALGNGSSLSVLEGQSLRLVCAVNSNPPARLSWTRGSLTLCPSRSS
 NPGLLELPRVHVRDEGEFTCRAQNAQGSQHISLSLSLQNEGTGTSRPVSQVTLAAVGGIE
 GRSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
 YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ
 ID NO:117)

Amino acid sequence of baboon Siglec-8 Domains 1, 2, and 3 Fusion Protein

MEGDRKYGDGYLLQVQELVTVQEGLCVHVPCSFSSYPKDDWTYSDPVHGYWFRAGDR
 PYQEAPVATNPNPDEVQAETQGRFQLLGDRWSNDCSLSINDARKGDEGSYFFRLERGR
 MKWSYKSQLNYKAKQLSVFVTALTQRPDILIQGTLES GHPRNLTCSPWACEQRMPPM
 ISWIGTSVSSLGPIARFSVLTLIPKPQDHGTSLTCQVTLPGTGVTTTTRTVQLDVSYPWN
 LTVTVFQGDDTASTALGNGSSLSVLEGQSLRLVCAVDSNPPARLSWTRGSLTLCPSQPW
 NPGLLELLRVHVKDEGEFTCQAENPRGSQHISLSLSLQNEGTGTARPVSEVTAAVGGIE
 GRSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
 YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ
 ID NO:118)

Amino acid sequence of baboon Siglec-8 Domains 1, 2, and 3

MEGDRKYGDGYLLQVQELVTVQEGLCVHVPCSFSSYPKDDWTYSDPVHGYWFRAGDR
 PYQEAPVATNPNPDEVQAETQGRFQLLGDRWSNDCSLSINDARKGDEGSYFFRLERGR
 MKWSYKSQLNYKAKQLSVFVTALTQRPDILIQGTLES GHPRNLTCSPWACEQRMPPM
 ISWIGTSVSSLGPIARFSVLTLIPKPQDHGTSLTCQVTLPGTGVTTTTRTVQLDVSYPWN
 LTVTVFQGDDTASTALGNGSSLSVLEGQSLRLVCAVDSNPPARLSWTRGSLTLCPSQPW
 NPGLLELLRVHVKDEGEFTCQAENPRGSQHISLSLSLQNEGTGTARPVSEVTAAVGG
 (SEQ ID NO:119)

VH-VL CrossMab on Anti Siglec-8 Arm + KiH

Anti-S6 LC (VL – CL)

QIVVTQSPATLSLSPGERATLSCTASSSVSSSYLHWYQQKPGQAPRLLIYSTSILASGIPAR
 FSGSGSGTDFTLTISLQPEDFAVYYCHQYHRSPYTFGQGTKLEIKRTVAAPSVFIFPPSD
 KKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLT
 LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:272)

Anti-S6 HC Knob (VH – CH1 – CH2 – CH3) no C-term lysine

QVQLQESGPGLVKPSSETLSLTCTVSGFSLTSYGVSWIRQPPGKGLEWIGVIWHDGSTSYH
 PSLKSRVTISRDTSKNQVSLKLSSVTAADTAVYYCASDGYSGTFAYWGQGLTVTVSSAS
 TKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
 YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDERVEPKSCDKTHTCPPCPAPELLGGPS
 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPCRE
 EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:273)

Anti-S6 HC Knob (VH – CH1 – CH2 – CH3) with C-term lysine

QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVSWIRQPPGKGLEWIGVIWHDGSTSYH
PSLKSRVTISRDTSKNQVSLKLSSVTAADTAVYYCASDGYSGTFAYWGQGLVTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDERVERPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRE
EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:274)

Anti-S8 CrossMab L (VH – CL)

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGQGT
TVTVSSRTVAAPSVFIFPPSDEELKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ
ID NO:275)

Anti-S8 CrossMab H Hole (VL – CH1 – CH2 – CH3) no C-term lysine

EIVLTQSPATLSLSPGERATLSCSATSSVSVMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSELEPEDFAVYYCQQRSSYPFTFGPGTKLDIKSSASTKGPSVFPLAPSS
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSREEMTKNQVSLSC
AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPG (SEQ ID NO:276)

Anti-S8 CrossMab H Hole (VL – CH1 – CH2 – CH3) with C-term lysine

EIVLTQSPATLSLSPGERATLSCSATSSVSVMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSELEPEDFAVYYCQQRSSYPFTFGPGTKLDIKSSASTKGPSVFPLAPSS
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSREEMTKNQVSLSC
AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:277)

VH-VL CrossMab on Anti Siglec-6 Arm + KiH

Anti-S8 LC (VL – CL)

EIVLTQSPATLSLSPGERATLSCSATSSVSVMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSELEPEDFAVYYCQQRSSYPFTFGPGTKLDIKRTVAAPSVFIFPPSDKK
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:278)

Anti-S8 HC Knob (VH – CH1 – CH2 – CH3) no C-term lysine

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGQGT

TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDERVERPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPG (SEQ ID NO:279)

Anti-S8 HC Knob (VH – CH1 – CH2 – CH3) with C-term lysine

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGSTNYSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYSMEYWGGQTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDERVERPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:280)

Anti-S6 CrossMab L (VH – CL)

QVQLQESGPGLVKPSSETLSLTCTVSGFSLTSYGVSWIRQPPGKGLEWIGVIWHDGSTSYHPSLKSRVTISRDTSKNQVSLKLSSVTAADTAVYYCASDGYSGTFAYWGQGLTVTVSSRTVAAPSVFIFPPSDEELKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:281)

Anti-S6 CrossMab H Hole (VL – CH1 – CH2 – CH3) no C-term lysine

QIVVTQSPATLSLSPGERATLSCTASSSVSSSYLHWYQQKPGQAPRLLIYSTSILASGIPARFSGSGSGTDFTLTISLQPEDFAVYYCHQYHRSPYTFGQGTKLEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPG (SEQ ID NO:282)

Anti-S6 CrossMab H Hole (VL – CH1 – CH2 – CH3) with C-term lysine

QIVVTQSPATLSLSPGERATLSCTASSSVSSSYLHWYQQKPGQAPRLLIYSTSILASGIPARFSGSGSGTDFTLTISLQPEDFAVYYCHQYHRSPYTFGQGTKLEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:283)

CH-CL CrossMab on Anti Siglec-8 Arm + KiH**Anti-S6 LC (VL – CL)**

QIVVTQSPATLSLSPGERATLSCTASSSVSSSYLHWYQQKPGQAPRLLIYSTSILASGIPAR
 FSGSGSGTDFLTITSSLPEDFAVYYCHQYHRSPYTFGQGKLEIKRTVAAPSVFIFPPSD
 EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLT
 LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:284)

Anti-S6 HC Knob (VH – CH1 – CH2 – CH3) no C-term lysine

QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVSWIRQPPGKGLEWIGVIWHDGSTSYH
 PSLKSRVTISRDTSKNQVSLKLSSVTAADTAVYYCASDGYSGTFAYWGQGLTVTVSSAS
 TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR
 EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD
 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:285)

Anti-S6 HC Knob (VH – CH1 – CH2 – CH3) with C-term lysine

QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVSWIRQPPGKGLEWIGVIWHDGSTSYH
 PSLKSRVTISRDTSKNQVSLKLSSVTAADTAVYYCASDGYSGTFAYWGQGLTVTVSSAS
 TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR
 EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD
 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:286)

Anti-S8 CrossMab L (VL – CH1)

EIVLTQSPATLSLSPGERATLSCSATSSVSVMHWFQQKPGQAPRLLIYSTSNLASGIPARF
 SGSGSGTDFLTITSSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIKSSASTKGPSVFPLAPSS
 KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVTVPSS
 SLGTQTYICNVNHKPSNTKVDKRVEPKSC (SEQ ID NO:287)

Anti-S8 CrossMab H Hole (VH – CL – CH2 – CH3) no C-term lysine

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
 NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYSMEYWGQGT
 TVTVSSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
 ESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECDKTHT
 CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
 GSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:288)

Anti-S8 CrossMab H Hole (VH – CL – CH2 – CH3) with C-term lysine

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
 NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYSMEYWGQGT
 TVTVSSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ

ESVTEQDSKDYSLSTLTLTKADYKHKVYACEVTHQGLSSPVTKSFNRGECDKTHT
 CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
 GSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:289)

CH-CL CrossMab on Anti Siglec-6 Arm + KiH

Anti-S8 LC (VL – CL)

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
 SGSGSGTDFTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIKRTVAAPSVFIFPPSDEQ
 LKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTL
 KADYKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:290)

Anti-S8 HC Knob (VH – CH1 – CH2 – CH3) no C-term lysine

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
 NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYSMEYWGQGT
 TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
 AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPA
 PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
 KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPG (SEQ ID NO:291)

Anti-S8 HC Knob (VH – CH1 – CH2 – CH3) with C-term lysine

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
 NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYSMEYWGQGT
 TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
 AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPA
 PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
 KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:292)

Anti-S6 CrossMab L (VL – CH1)

QIVVTQSPATLSLSPGERATLSCTASSSVSSSYLHWYQQKPGQAPRLLIYSTSILASGIPAR
 FSGSGSGTDFTLTISSLQPEDFAVYYCHQYHRSPYTFGQGTKLEIKSSASTKGPSVFPLAP
 SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
 SSSLGTQTYICNVNHKPSNTKVDKRVEPKSC (SEQ ID NO:293)

Anti-S6 CrossMab H Hole (VH – CL – CH2 – CH3) no C-term lysine

QVQLQESGPGLVKPSSETLSLTCTVSGFSLTSYGVSWIRQPPGKGLEWIGVIWHDGSTSYH
 PSLKSRVTISRDTSKNQVSLKLSSVTAADTAVYYCASDGYSGTFAYWGQGTLVTVSSRT
 VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDS
 KDSTYSLSTLTLTKADYKHKVYACEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPE
 LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVC

TLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:294)

Anti-S6 CrossMab H Hole (VH – CL – CH2 – CH3) with C-term lysine

QVQLQESGPGLVKPSSETLSLTCTVSGFSLTSYGVSWIRQPPGKGLEWIGVIWHDGSTSYHPSLKSRVTISRDTSKNQVSLKLSSVTAADTAVYYCASDGYSGTFAYWGQGLTVTVSSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:295)

DuetMab on Anti Siglec-6 Arm + KiH

Anti-S6 LC (VL – CL)

QIVVTQSPATLSLSPGERATLSCTASSSVSSSYLHWYQQKPGQAPRLLIYSTSILASGIPARFSGSGSGTDFTLTISLQPEDFAVYYCHQYHRSPYTFGQGTKLEIKRTVAAPSVFIFPPSEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:284)

Anti-S6 HC Knob (VH – CH1 – CH2 – CH3) no C-term lysine

QVQLQESGPGLVKPSSETLSLTCTVSGFSLTSYGVSWIRQPPGKGLEWIGVIWHDGSTSYHPSLKSRVTISRDTSKNQVSLKLSSVTAADTAVYYCASDGYSGTFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:285)

Anti-S6 HC Knob (VH – CH1 – CH2 – CH3) with C-term lysine

QVQLQESGPGLVKPSSETLSLTCTVSGFSLTSYGVSWIRQPPGKGLEWIGVIWHDGSTSYHPSLKSRVTISRDTSKNQVSLKLSSVTAADTAVYYCASDGYSGTFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:286)

Anti-S8 DuetMab LC (VL – CL)

EIVLTQSPATLSLSPGERATLSCSATSSVSVMHWFQQKPGQAPRLLIYSTSNLASGIPARFSGSGSGTDFTLTISLQPEDFAVYYCQQRSSYPFTFGPGTKLDIKRTVAAPSVFIFPPCDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEV (SEQ ID NO:298)

Anti-S8 HC DuetMab Hole (VH – CH1 – CH2 – CH3) no C-term lysine

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGQGT
TVTVSSASTKGPSVCPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSVDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFL
VSKLTVDKSRWQQGNVFSCSVMHEALHNRFTQKSLSLSPG (SEQ ID NO:299)

Anti-S8 HC DuetMab Hole (VH – CH1 – CH2 – CH3) with C-term lysine

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIIYGAHWVRQAPGKGLEWVGVIWAGGST
NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSM EYWGQGT
TVTVSSASTKGPSVCPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSVDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFL
VSKLTVDKSRWQQGNVFSCSVMHEALHNRFTQKSLSLSPGK (SEQ ID NO:300)

CLAIMS

What is claimed is:

1. A bispecific antibody comprising a first antigen binding domain that binds to human Siglec-6 and a second antigen binding domain that binds to human Siglec-8.
2. The bispecific antibody of claim 1, wherein the first antigen binding domain is a humanized antigen binding domain that binds to Domain 1 of an extracellular domain of human Siglec-6.
3. The bispecific antibody of claim 2, wherein Domain 1 of the extracellular domain of human Siglec-6 comprises the amino acid sequence of SEQ ID NO:121.
4. The bispecific antibody of claim 1, wherein the first antigen binding domain binds to Domain 2 of an extracellular domain of human Siglec-6.
5. The bispecific antibody of claim 4, wherein Domain 2 of the extracellular domain of human Siglec-6 comprises the amino acid sequence of SEQ ID NO:122.
6. The bispecific antibody of claim 1, wherein the first antigen binding domain binds to Domain 3 of an extracellular domain of human Siglec-6.
7. The bispecific antibody of claim 6, wherein Domain 3 of the extracellular domain of human Siglec-6 comprises the amino acid sequence of SEQ ID NO:123.
8. The bispecific antibody of any one of claims 4-7, wherein the first antigen binding domain is a humanized antigen binding domain.
9. The bispecific antibody of any one of claims 1-8, wherein the first antigen binding domain comprises a first heavy chain variable (VH) region and a first light chain variable (VL) region.
10. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:124, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:125, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:126; and wherein the first VL region comprises an HVR-L1 comprising the amino acid

sequence of SEQ ID NO:127, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:128, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:129.

11. The bispecific antibody of claim 10, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:254, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:255.

12. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:208, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:209, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:210; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:211, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:212, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:213.

13. The bispecific antibody of claim 12, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:260, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:261.

14. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:202, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:203, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:204; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:205, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:206, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:207.

15. The bispecific antibody of claim 14, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:256, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:257.

16. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:130, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:131, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:132; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:133, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:134, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:135.

17. The bispecific antibody of claim 16, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:252, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:253.
18. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:196, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:197, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:198; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:199, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:200, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:201.
19. The bispecific antibody of claim 18, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:250, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:251.
20. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:136, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:137, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:138; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:139, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:140, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:141.
21. The bispecific antibody of claim 20, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:258, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:259.
22. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:190, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:191, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:192; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:193, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:194, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:195.

23. The bispecific antibody of claim 22, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:248, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:249.
24. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:172, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:173, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:174; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:175, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:176, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:177.
25. The bispecific antibody of claim 24, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:242, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:243.
26. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:148, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:149, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:150; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:151, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:152, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:153.
27. The bispecific antibody of claim 26, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:240, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:241.
28. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:142, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:143, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:144; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:145, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:146, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:147.

29. The bispecific antibody of claim 28, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:234, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:235.

30. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:154, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:155, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:156; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:157, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:158, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:159.

31. The bispecific antibody of claim 30, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:232, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:233.

32. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:160, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:161, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:162; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:163, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:164, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:165.

33. The bispecific antibody of claim 32, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:236, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:237.

34. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:166, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:167, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:168; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:169, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:170, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:171.

35. The bispecific antibody of claim 34, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:238, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:239.

36. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:178, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:179, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:180; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:181, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:182, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:183.

37. The bispecific antibody of claim 36, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:244, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:245.

38. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:184, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:185, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:186; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:187, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:188, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:189.

39. The bispecific antibody of claim 38, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:246, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:247.

40. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:226, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:227, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:228; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:229, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:230, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:231.

41. The bispecific antibody of claim 40, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:266, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:267.
42. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:214, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:215, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:216; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:217, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:218, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:219.
43. The bispecific antibody of claim 42, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:262, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:263.
44. The bispecific antibody of claim 9, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:220, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:221, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:222; and wherein the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:223, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:224, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:225.
45. The bispecific antibody of claim 44, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:264, and wherein the first VL region comprises the amino acid sequence of SEQ ID NO:265.
46. The bispecific antibody of any one of claims 1-45, wherein the second antigen binding domain is a humanized antigen binding domain with a greater binding affinity or avidity to human Siglec-8 than the binding affinity or avidity of antibody 2E2 or 2C4 to human Siglec-8.
47. The bispecific antibody of any one of claims 1-46, wherein the second antigen binding domain comprises a second heavy chain variable (VH) region and a second light chain variable (VL) region.

48. The bispecific antibody of claim 47, wherein the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and wherein the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66.

49. The bispecific antibody of claim 47, wherein the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence selected from SEQ ID NOs:67-70; and wherein the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:71.

50. The bispecific antibody of claim 47, wherein the second VH region comprises the amino acid sequence of SEQ ID NO:6; and the second VL region comprises the amino acid sequence of SEQ ID NO:16 or 21.

51. The bispecific antibody of claim 47, wherein the second VH region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:11-14; and the second VL region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:23-24.

52. The bispecific antibody of claim 47, wherein the second VH region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:2-14; and the second VL region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:16-24.

53. The bispecific antibody of claim 47, wherein the second VH region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:2-10; and the second VL region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:16-22.

54. The bispecific antibody of claim 47, wherein:

(a) the second VH region comprises:

- (1) an HC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:26-29;
- (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61;
- (3) an HC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:31-36;
- (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62;
- (5) an HC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:38-43;
- (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and
- (7) an HC-FR4 comprising the amino acid sequence selected from SEQ ID NOs:45-46, and

(b) the second VL region comprises:

- (1) an LC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:48-49;
- (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64;
- (3) an LC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:51-53;
- (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65;
- (5) an LC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:55-58;
- (6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and
- (7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60.

55. The bispecific antibody of claim 47, wherein:

(a) the second VH region comprises:

- (1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:26;
- (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61;
- (3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:34;
- (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62;
- (5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:38;
- (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and

(7) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:45; and
(b) the second VL region comprises:

- (1) an LC-FR1 comprising the amino acid sequence of SEQ ID NO:48;
- (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64;
- (3) an LC-FR2 comprising the amino acid sequence of SEQ ID NO:51;
- (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65;
- (5) an LC-FR3 comprising the amino acid sequence of SEQ ID NO:55;
- (6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and
- (7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60.

56. The bispecific antibody of claim 47, wherein:

(a) the second VH region comprises:

- (1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:26;
- (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61;
- (3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:34;
- (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62;
- (5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:38;
- (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and
- (7) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:45; and

(b) the second VL region comprises:

- (1) an LC-FR1 comprising the amino acid sequence of SEQ ID NO:48;
- (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64;
- (3) an LC-FR2 comprising the amino acid sequence of SEQ ID NO:51;
- (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65;
- (5) an LC-FR3 comprising the amino acid sequence of SEQ ID NO:58;
- (6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and
- (7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60.

57. The bispecific antibody of claim 47, wherein:

the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:88, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:91, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:94; and the second VL region

comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:97, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:100, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:103;

the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:89, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:92, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:95; and the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:98, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:101, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:104; or

the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:90, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:93, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:96; and the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:99, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:102, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:105.

58. The bispecific antibody of claim 47, wherein:

the second VH region comprises the amino acid sequence of SEQ ID NO:106; and/or the second VL region comprises the amino acid sequence of SEQ ID NO:109;

the second VH region comprises the amino acid sequence of SEQ ID NO:107; and/or the second VL region comprises the amino acid sequence of SEQ ID NO:110; or

the second VH region comprises the amino acid sequence of SEQ ID NO:108; and/or the second VL region comprises the amino acid sequence of SEQ ID NO:111.

59. The bispecific antibody of any one of claims 1-58, wherein the first antigen binding domain is a human, humanized, or chimeric antigen binding domain.

60. The bispecific antibody of any one of claims 1-59, wherein the second antigen binding domain is a human, humanized, or chimeric antigen binding domain.

61. The bispecific antibody of claim 9 or claim 47, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:124, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:125, and an HVR-H3 comprising the amino acid sequence

of SEQ ID NO:126; the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:127, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:128, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:129; the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66.

62. The bispecific antibody of claim 61, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:254, the first VL region comprises the amino acid sequence of SEQ ID NO:255, the second VH region comprises the amino acid sequence of SEQ ID NO:6, and the second VL region comprises the amino acid sequence of SEQ ID NO:16 or 21.

63. The bispecific antibody of claim 9 or claim 47, wherein the first VH region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:208, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:209, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:210; the first VL region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:211, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:212, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:213; the second VH region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and the second VL region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66.

64. The bispecific antibody of claim 63, wherein the first VH region comprises the amino acid sequence of SEQ ID NO:260, the first VL region comprises the amino acid sequence of SEQ ID NO:261, the second VH region comprises the amino acid sequence of SEQ ID NO:6, and the second VL region comprises the amino acid sequence of SEQ ID NO:16 or 21.

65. The bispecific antibody of any one of claims 1-64, wherein the bispecific antibody comprises an Fc region.
66. The bispecific antibody of claim 65, wherein the Fc region is a human IgG Fc region.
67. The bispecific antibody of claim 66, wherein the Fc region is a human IgG1 Fc region.
68. The bispecific antibody of claim 67, wherein the human IgG1 Fc region is non-fucosylated.
69. The bispecific antibody of claim 66, wherein the Fc region is a human IgG1 Fc region.
70. The bispecific antibody of claim 66, wherein the Fc region is a human IgG4 Fc region.
71. The bispecific antibody of claim 70, wherein the human IgG4 Fc region comprises the amino acid substitution S228P, wherein the amino acid residues are numbered according to the EU index as in Kabat.
72. The bispecific antibody of any one of claims 65-71, wherein the bispecific antibody has been engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity.
73. The bispecific antibody of claim 72, wherein the bispecific antibody comprises at least one amino acid substitution in the Fc region that improves ADCC activity.
74. The bispecific antibody of any one of claims 65-73, wherein at least one or two of the heavy chains of the antibody is non-fucosylated.
75. The bispecific antibody of any one of claims 65-74, wherein the bispecific antibody comprises a first antibody heavy chain comprising a first VH region and a first Fc region, a first antibody light chain comprising a first VL region, a second antibody heavy chain comprising a second VH region and a second Fc region, and a second antibody light chain comprising a second VL region; wherein the first VH and VL regions form the first antigen binding domain; and wherein the second VH and VL regions form the second antigen binding domain.
76. The bispecific antibody of claim 75, wherein the first antibody heavy chain comprises a first constant heavy chain (CH1) region that comprises a substitution of a native cysteine to a non-cysteine amino acid and a substitution of a native non-cysteine amino acid to a cysteine

amino acid; wherein the first antibody light chain comprises a constant light chain (CL) region that comprises a substitution of a native cysteine to a non-cysteine amino acid and a substitution of a native non-cysteine amino acid to a cysteine amino acid; and wherein the substituted cysteines on the CH1 region and the CL region of the first antibody heavy and light chains, respectively, form a disulfide bond.

77. The bispecific antibody of claim 76, wherein the CH1 region of the first antibody heavy chain comprises F126C and C220V substitutions, and wherein the CL region of the first antibody light chain comprises S121C and C214V substitutions, wherein the amino acid residues are numbered according to the EU index as in Kabat.

78. The bispecific antibody of claim 75, wherein the second antibody heavy chain comprises a first constant heavy chain (CH1) region that comprises a substitution of a native cysteine to a non-cysteine amino acid and a substitution of a native non-cysteine amino acid to a cysteine amino acid; wherein the second antibody light chain comprises a constant light chain (CL) region that comprises a substitution of a native cysteine to a non-cysteine amino acid and a substitution of a native non-cysteine amino acid to a cysteine amino acid; and wherein the substituted cysteines on the CH1 region and the CL region of the second antibody heavy and light chains, respectively, form a disulfide bond.

79. The bispecific antibody of claim 78, wherein the CH1 region of the second antibody heavy chain comprises F126C and C220V substitutions, and wherein the CL region of the second antibody light chain comprises S121C and C214V substitutions, wherein the amino acid residues are numbered according to the EU index as in Kabat.

80. The bispecific antibody of any one of claims 75-79, wherein the first antibody heavy chain comprises a constant light chain (CL) region that replaces a native first constant heavy chain (CH1) region, and wherein the first antibody light chain comprises a CH1 region that replaces a native CL region.

81. The bispecific antibody of any one of claims 75-79, wherein the second antibody heavy chain comprises a constant light chain (CL) region that replaces a native first constant heavy chain (CH1) region, and wherein the second antibody light chain comprises a CH1 region that replaces a native CL region.

82. The bispecific antibody of any one of claims 75-79, wherein the first antibody heavy chain comprises a CH1 region comprising substitution of one or more native, non-positively charged amino acid(s) to positively charged amino acid(s); wherein the first antibody light chain comprises a CL region comprising substitution of one or more native, non-negatively charged amino acid(s) to negatively charged amino acid(s); wherein the first VL region replaces the first VH region on the first antibody heavy chain; wherein the first VH region replaces the first VL region on the first antibody light chain; wherein the second antibody heavy chain comprises a CH1 region comprising substitution of one or more native, non-negatively charged amino acid(s) to negatively charged amino acid(s); and wherein the second antibody light chain comprises a CL region comprising substitution of one or more native, non-positively charged amino acid(s) to positively charged amino acid(s).

83. The bispecific antibody of claim 82, wherein the first antibody light chain comprises a Q124E substitution; wherein the second antibody light chain comprises E123K and Q124K substitutions; and wherein the second antibody heavy chain comprises K147E and K213E substitutions; wherein the amino acid residues are numbered according to the EU index as in Kabat.

84. The bispecific antibody of any one of claims 75-79, wherein the second antibody heavy chain comprises a CH1 region comprising substitution of one or more native, non-positively charged amino acid(s) to positively charged amino acid(s); wherein the second antibody light chain comprises a CL region comprising substitution of one or more native, non-negatively charged amino acid(s) to negatively charged amino acid(s); wherein the second VL region replaces the second VH region on the second antibody heavy chain; wherein the second VH region replaces the second VL region on the second antibody light chain; wherein the first antibody heavy chain comprises a CH1 region comprising substitution of one or more native, non-negatively charged amino acid(s) to negatively charged amino acid(s); and wherein the first antibody light chain comprises a CL region comprising substitution of one or more native, non-positively charged amino acid(s) to positively charged amino acid(s).

85. The bispecific antibody of claim 84, wherein the second antibody light chain comprises a Q124E substitution; wherein the first antibody light chain comprises E123K and Q124K substitutions; and wherein the first antibody heavy chain comprises K147E and K213E

substitutions; wherein the amino acid residues are numbered according to the EU index as in Kabat.

86. The bispecific antibody of any one of claims 65-85, wherein the first antibody heavy chain comprises one or more substitutions encoding a protuberance, wherein the second antibody heavy chain comprises one or more substitutions encoding a cavity, and wherein the protuberance of the first antibody heavy chain is positionable in the cavity of the second antibody heavy chain.

87. The bispecific antibody of claim 86, wherein the first antibody heavy chain comprises T366W and S354C substitutions and wherein the second antibody heavy chain comprises Y349C, T366S, L368A, and Y407V substitutions, wherein the amino acid residues are numbered according to the EU index as in Kabat.

88. The bispecific antibody of any one of claims 65-85, wherein the second antibody heavy chain comprises one or more substitutions encoding a protuberance, wherein the first antibody heavy chain comprises one or more substitutions encoding a cavity, and wherein the protuberance of the second antibody heavy chain is positionable in the cavity of the first antibody heavy chain.

89. The bispecific antibody of claim 88, wherein the second antibody heavy chain comprises T366W and S354C substitutions and wherein the first antibody heavy chain comprises Y349C, T366S, L368A, and Y407V substitutions, wherein the amino acid residues are numbered according to the EU index as in Kabat.

90. The bispecific antibody of any one of claims 65-89, wherein the first antibody heavy chain comprises H435R and Y436F substitutions, wherein the amino acid residues are numbered according to the EU index as in Kabat.

91. The bispecific antibody of any one of claims 65-89, wherein the second antibody heavy chain comprises H435R and Y436F substitutions, wherein the amino acid residues are numbered according to the EU index as in Kabat.

92. The bispecific antibody of any one of claims 1-9 and 47, wherein the bispecific antibody comprises a first antibody light chain comprising the amino acid sequence of SEQ ID NO:272, a

first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:273 or 274, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:275, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:276 or 277.

93. The bispecific antibody of any one of claims 1-9 and 47, wherein the bispecific antibody comprises a first antibody light chain comprising the amino acid sequence of SEQ ID NO:281, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:282 or 283, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:278, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:279 or 280.

94. The bispecific antibody of any one of claims 1-9 and 47, wherein the bispecific antibody comprises a first antibody light chain comprising the amino acid sequence of SEQ ID NO:284, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:285 or 286, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:287, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:288 or 289.

95. The bispecific antibody of any one of claims 1-9 and 47, wherein the bispecific antibody comprises a first antibody light chain comprising the amino acid sequence of SEQ ID NO:293, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:294 or 295, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:290, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:291 or 292.

96. The bispecific antibody of any one of claims 1-9 and 47, wherein the bispecific antibody comprises a first antibody light chain comprising the amino acid sequence of SEQ ID NO:284, a first antibody heavy chain comprising the amino acid sequence of SEQ ID NO:285 or 286, a second antibody light chain comprising the amino acid sequence of SEQ ID NO:298, and a second antibody heavy chain comprising the amino acid sequence of SEQ ID NO:299 or 300.

97. The bispecific antibody of any one of claims 1-96, wherein binding of the bispecific antibody to an extracellular domain of human Siglec-6 when expressed on a surface of a human mast cell leads to a reduced level of Siglec-6 on the cell surface.

98. The bispecific antibody of any one of claims 1-97, wherein binding of the bispecific antibody to an extracellular domain of human Siglec-8 when expressed on a surface of a human mast cell leads to a reduced level of Siglec-8 on the cell surface.

99. The bispecific antibody of any one of claims 1-98, wherein binding of the bispecific antibody to an extracellular domain of human Siglec-6 inhibits activation of a mast cell that expresses the human Siglec-6.

100. The bispecific antibody of any one of claims 1-99, wherein binding of the bispecific antibody to an extracellular domain of human Siglec-8 inhibits activation of a mast cell that expresses the human Siglec-8.

101. The bispecific antibody of any one of claims 1-100, wherein binding of the bispecific antibody to an extracellular domain of human Siglec-8 depletes eosinophils expressing human Siglec-8 *in vivo*.

102. A composition comprising the bispecific antibody of any one of claims 1-101.

103. The composition of claim 102, wherein the bispecific antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than 50% of the N-glycoside-linked carbohydrate chains contain a fucose residue.

104. The composition of claim 103, wherein substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue.

105. A pharmaceutical composition comprising the bispecific antibody of any one of claims 1-101 and a pharmaceutically acceptable carrier.

106. A polynucleotide encoding the bispecific antibody of any one of claims 1-101.

107. A kit of polynucleotides comprising a first polynucleotide encoding the first antibody heavy chain and first antibody light chain of any one of claims 1-101 and a second polynucleotide encoding the second antibody heavy chain and second antibody light chain of any one of claims 1-101.

108. A vector comprising the polynucleotide of claim 106.

109. A kit of vectors comprising a first vector comprising a polynucleotide encoding the first antibody heavy chain and first antibody light chain of any one of claims 1-101 and a second

vector comprising a second polynucleotide encoding the second antibody heavy chain and second antibody light chain of any one of claims 1-101.

110. A host cell comprising the polynucleotide of claim 106, the vector of claim 108, or the kit of claim 107 or claim 109.

111. The host cell of claim 110, wherein the host cell is a mammalian or insect cell.

112. The host cell of claim 111, wherein the host cell is Chinese hamster ovary (CHO) cell.

113. The host cell of claim 111 or claim 112, wherein the host cell comprises a Fut8 knockout.

114. The host cell of claim 111 or claim 112, wherein the host cell overexpresses GnT-III.

115. The host cell of claim 114, wherein the host cell additionally overexpresses ManII.

116. A method of producing a bispecific antibody, comprising culturing the host cell of any one of claims 110-115 under a condition that produces the bispecific antibody.

117. The method of claim 116, further comprising recovering the bispecific antibody produced by the host cell.

118. A bispecific antibody produced by the method of claim 116 or claim 117.

119. A method of treating a disease or condition characterized by increased activity and/or number of mast cells expressing Siglec-6 and/or Siglec-8 in a subject, comprising administering to the subject an effective amount of the bispecific antibody of any one of claims 1-101 and 118 or the composition of any one of claims 102-105.

120. A method of inhibiting activation of mast cells expressing Siglec-6 and/or Siglec-8 in a subject in need thereof, comprising administering to the subject an effective amount of the bispecific antibody of any one of claims 1-101 and 118 or the composition of any one of claims 102-105.

121. A method of depleting mast cells expressing Siglec-6 and/or Siglec-8 in a subject in need thereof, comprising administering to the subject an effective amount of the bispecific antibody of any one of claims 1-101 and 118 or the composition of any one of claims 102-105.

122. The method of any one of claims 119-121, wherein administration of the bispecific antibody or composition results in a decrease in one or more symptoms in the subject, as compared to the one or more symptoms in the subject prior to the administration.

123. The method of claim 122, wherein the one or more symptoms are selected from the group consisting of nausea, cramping, constipation, abdominal pain, bloating, vomiting, diarrhea, fatigue, eye pain, light sensitivity, redness, discharge, runny nose, headache, dizziness, brain fog, itching, flushing, sweating, hives, hypotension, shortness of breath, bone pain, joint pain, weight loss, osteoporosis, angioedema, chest pain, anxiety, depression, rapid heartbeat, bronchoconstriction, and general pain.

124. The method of claim 119, wherein the disease or condition is mastocytosis, mast cell leukemia, mast cell activation syndrome, gastroparesis, osteoporosis, osteopenia, renal osteodystrophy, bone fracture, Alzheimer's disease, chronic neuropathic pain, hyperalgesia, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), graft vs. host disease (GVH), colitis, hereditary alpha tryptasemia, neurofibroma, Kounis syndrome, urticaria, atopic dermatitis, contact dermatitis, angioedema, prurigo nodularis, cholangitis, psoriasis, irritable bowel syndrome (IBS), functional dyspepsia, asthma, allergy, keloid, chronic rhinosinusitis, aspirin exacerbated respiratory disease (AERD), chronic obstructive pulmonary disease (COPD), bullous pemphigoid, idiopathic pulmonary fibrosis, systemic sclerosis, interstitial cystitis, hidradenitis suppurativa, alopecia areata, vitiligo, mast cell gastrointestinal disease, Crohn's disease, rheumatoid arthritis, gastroesophageal reflux disease, viral infection, achalasia, postural tachycardia syndrome, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), complex regional pain syndrome, osteoarthritis, or Ehlers-Danlos syndrome.

125. The method of any one of claims 119-124, wherein the subject has or has been diagnosed with mastocytosis, mast cell leukemia, mast cell activation syndrome, gastroparesis, osteoporosis, osteopenia, renal osteodystrophy, bone fracture, Alzheimer's disease, chronic neuropathic pain, hyperalgesia, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), graft vs. host disease (GVH), colitis, hereditary alpha tryptasemia,

neurofibroma, Kounis syndrome, urticaria, atopic dermatitis, contact dermatitis, angioedema, prurigo nodularis, cholangitis, psoriasis, irritable bowel syndrome (IBS), functional dyspepsia, asthma, allergy, keloid, chronic rhinosinusitis, aspirin exacerbated respiratory disease (AERD), chronic obstructive pulmonary disease (COPD), bullous pemphigoid, idiopathic pulmonary fibrosis, systemic sclerosis, interstitial cystitis, hidradenitis suppurativa, alopecia areata, vitiligo, mast cell gastrointestinal disease, Crohn's disease, rheumatoid arthritis, gastroesophageal reflux disease, viral infection, achalasia, postural tachycardia syndrome, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), complex regional pain syndrome, osteoarthritis, or Ehlers-Danlos syndrome.

126. A method of treating a disease or condition mediated by cells expressing Siglec-8 in a subject, comprising administering to the subject an effective amount of the bispecific antibody of any one of claims 1-101 and 118 or the composition of any one of claims 102-105.

127. The method of claim 126, wherein the disease is an eosinophil mediated-disease.

128. The method of claim 126, wherein the disease is a mast cell mediated-disease.

129. The method of claim 126, wherein the disease is selected from the group consisting of allergic rhinitis, nasal polyposis, pauci granulocytic asthma, acute or chronic airway hypersensitivity, eosinophilic esophagitis, eosinophilic leukemia, Churg-Strauss syndrome, inflammation associated with a cytokine, inflammation associated with cells expressing Siglec-8, malignancy associated with cells expressing Siglec-8, physical urticaria, cold urticaria, pressure-urticaria, bullous pemphigoid, food allergy, chronic rhinosinusitis with concomitant asthma, aspirin-exacerbated respiratory disease, adult onset non-atopic asthma with sinus disease, chronic obstructive pulmonary disease, fibrotic disease, pre-fibrotic disease, mastocytosis, advanced systemic mastocytosis, indolent systemic mastocytosis (ISM), inflammatory bowel disease (IBD), eosinophilic esophagitis (EOE), eosinophilic gastritis (EG), eosinophilic gastroenteritis (EGE), eosinophilic colitis (EOC), eosinophilic duodenitis, mast cell gastritis or mast cell gastroenteritis, gastritis or gastroenteritis with elevated mast cells, irritable bowel syndrome, irritable bowel syndrome with elevated mast cells, functional gastrointestinal disease, functional dyspepsia, allergic conjunctivitis, giant papillary conjunctivitis, chronic urticaria, allergic bronchopulmonary aspergillosis (ABPA), allergic asthma, asthma with eosinophil or mast cell phenotype, eosinophilic granulomatosis with polyangiitis (EGPA), celiac

disease, gastroparesis, hypereosinophilic syndrome, atopic dermatitis, anaphylaxis, angioedema, mast cell activation syndrome/disorder, and eosinophilic fasciitis.

130. The method of any one of claims 119-129, wherein the subject is a human.

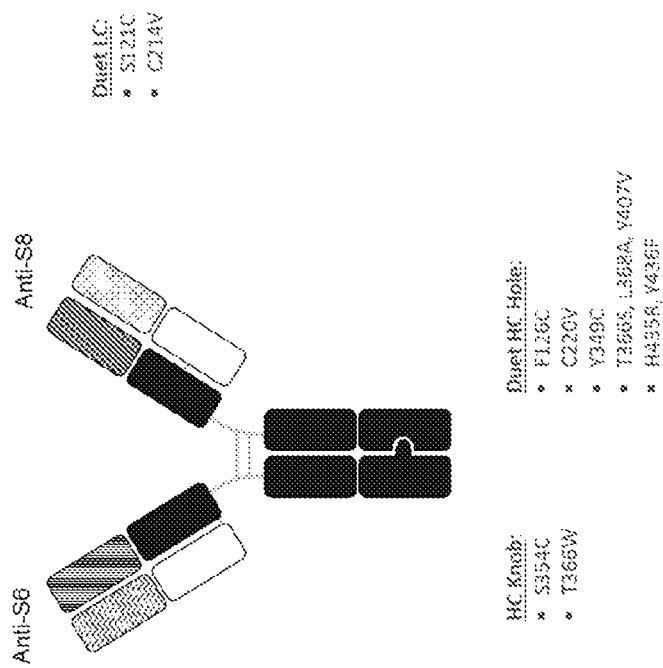


FIG. 1A

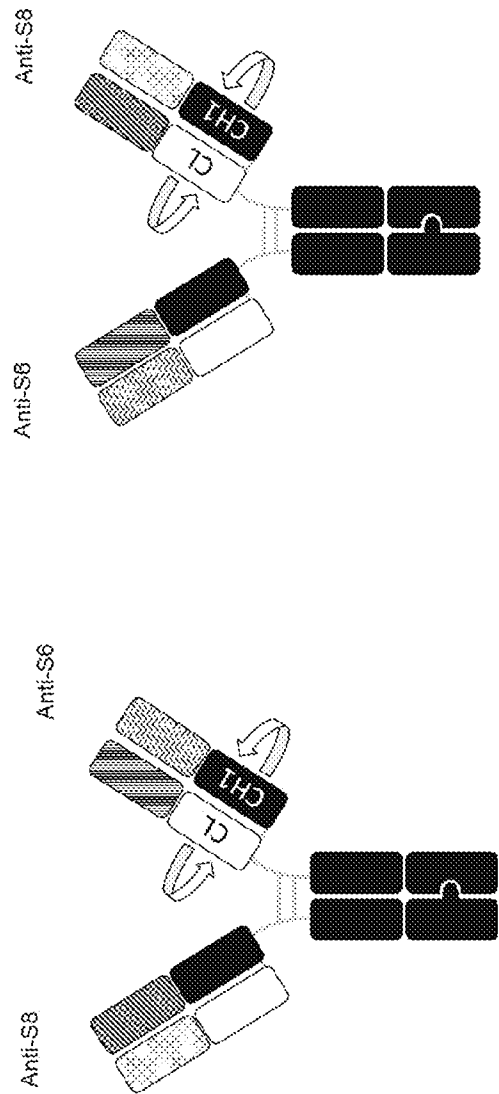


FIG. 1B

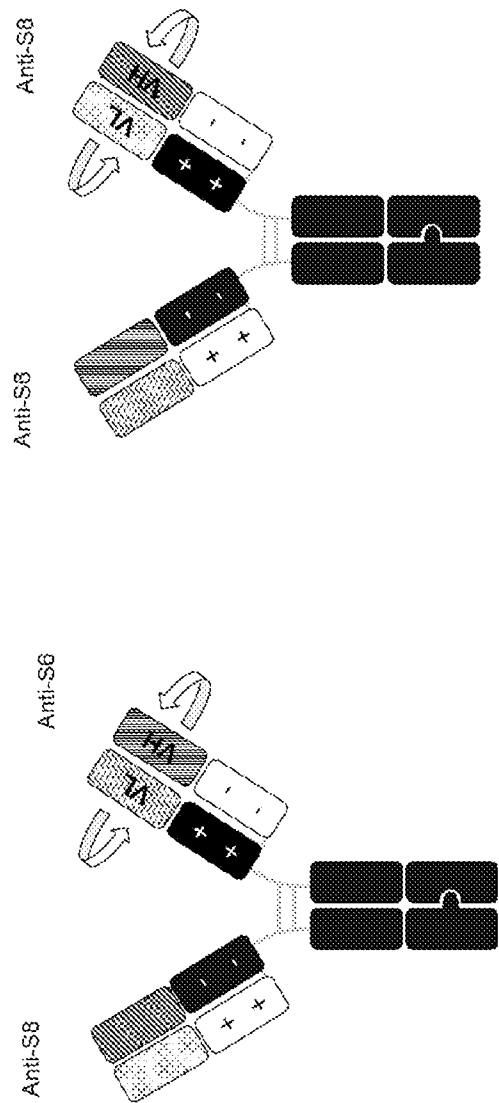


FIG. 1C

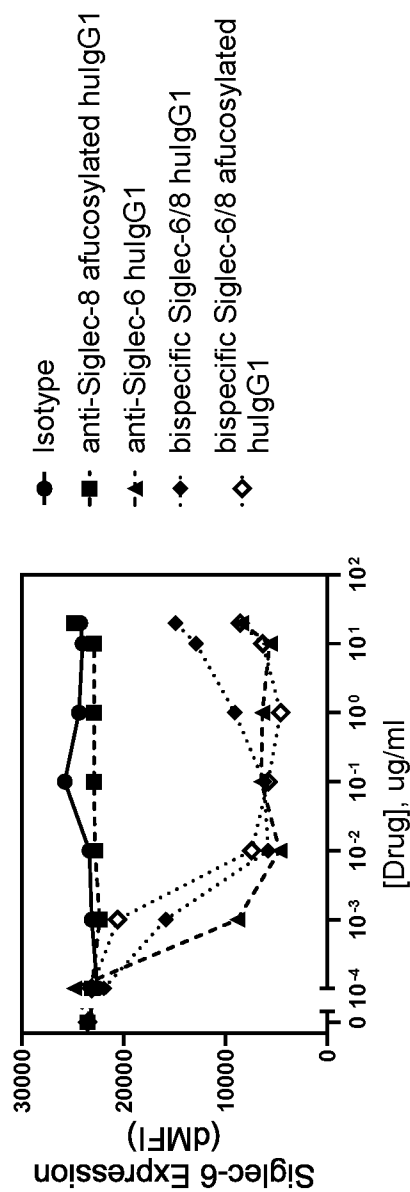


FIG. 2A

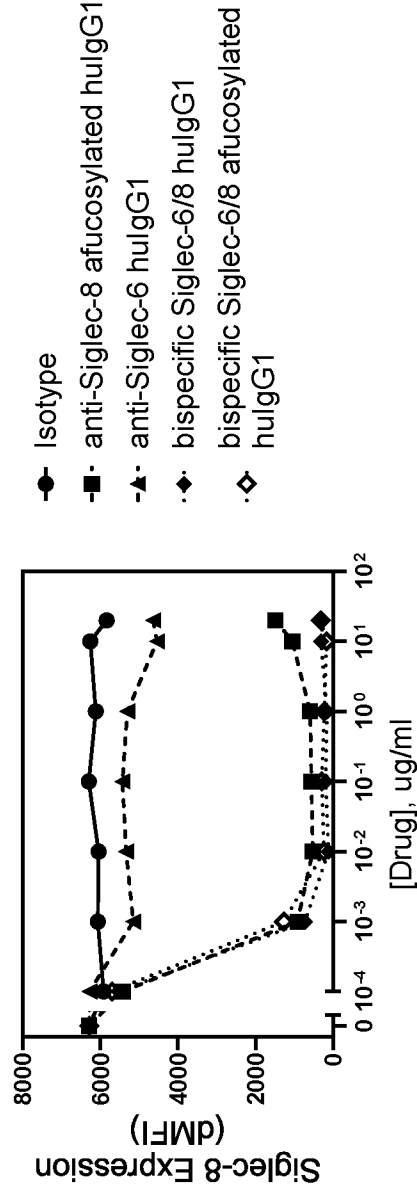


FIG. 2B

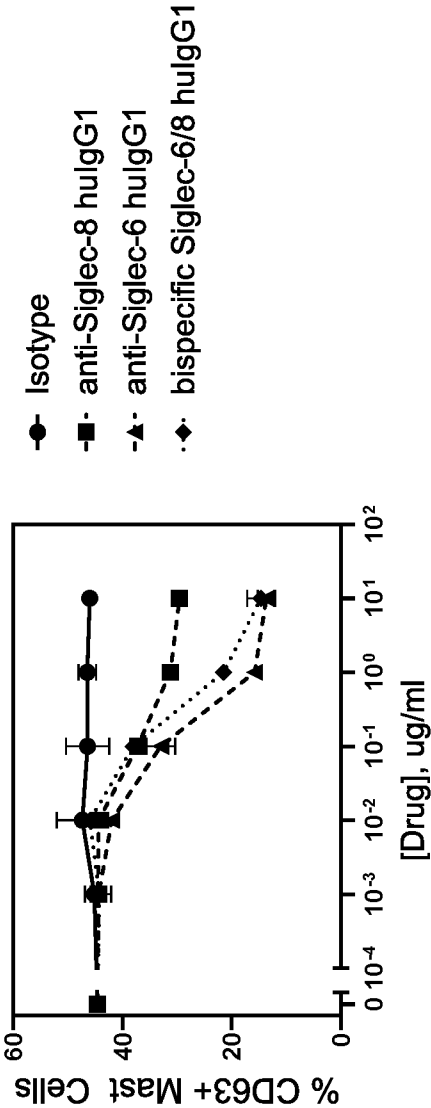


FIG. 3

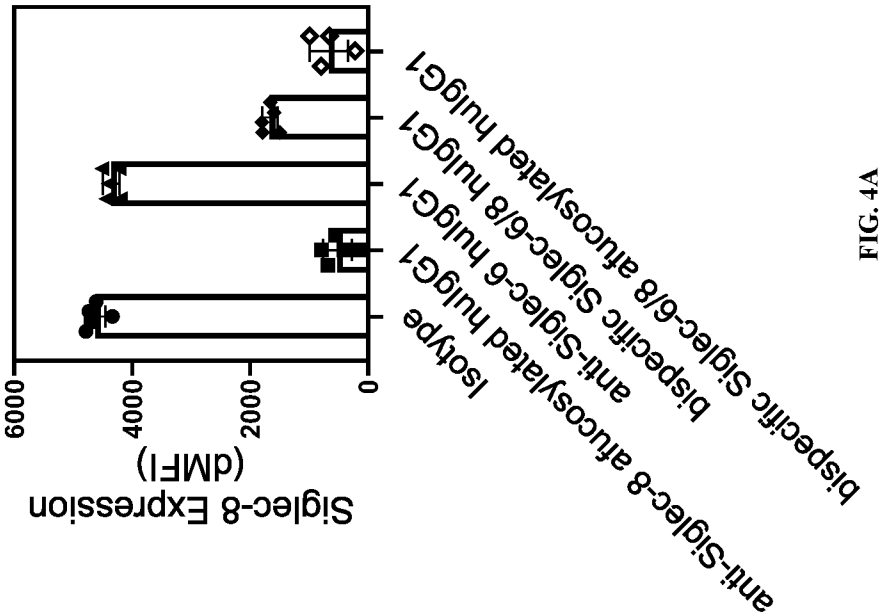


FIG. 4A

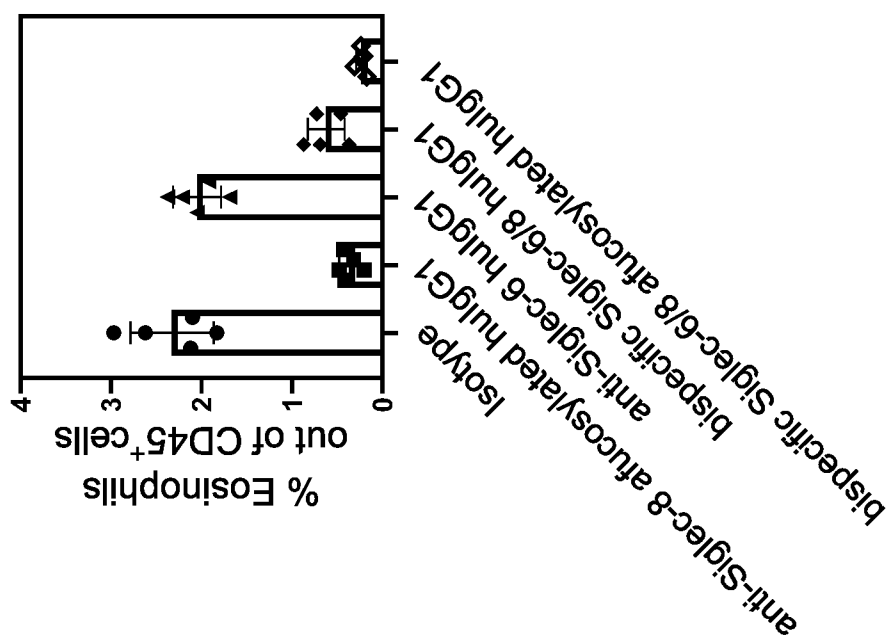


FIG. 4B

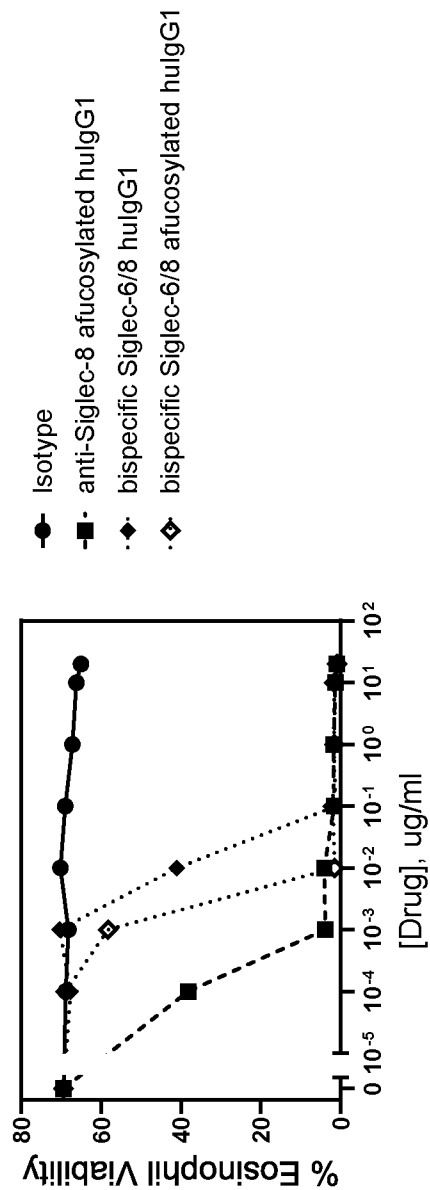


FIG. 4C

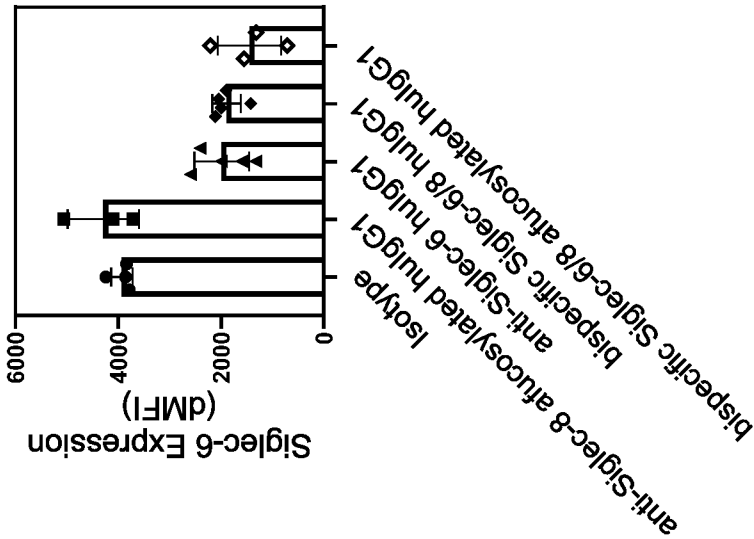


FIG. 5A

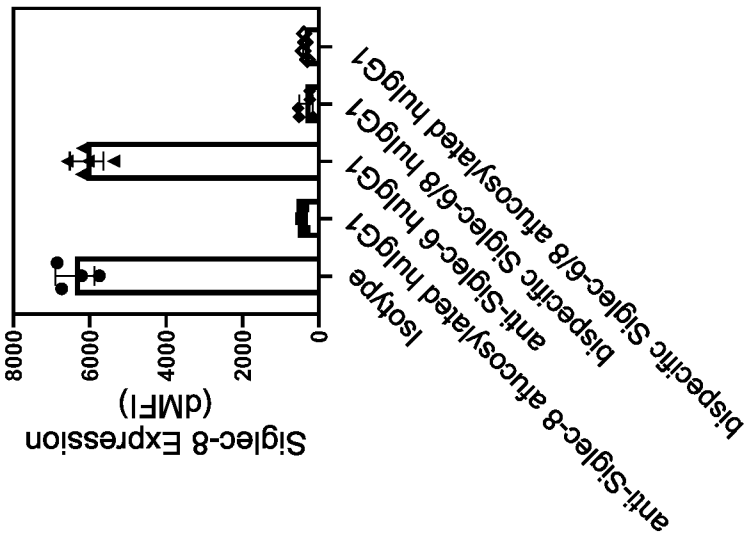


FIG. 5B

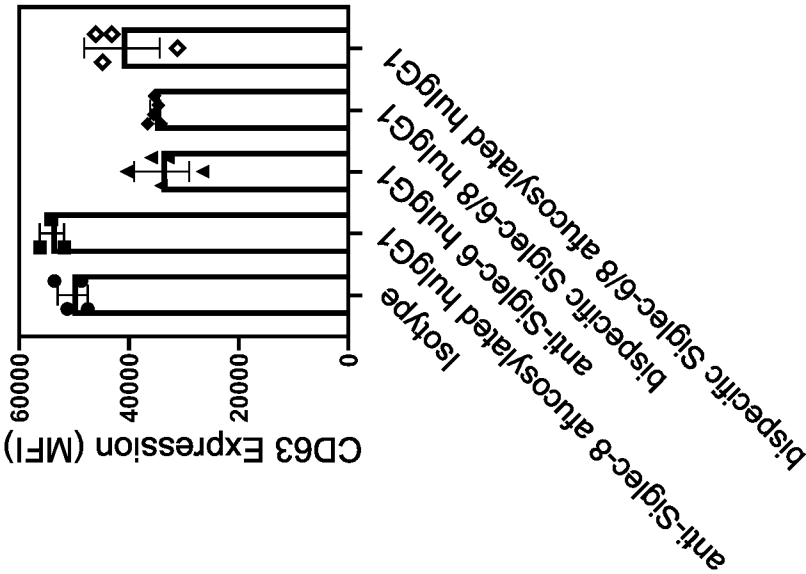


FIG. 6A

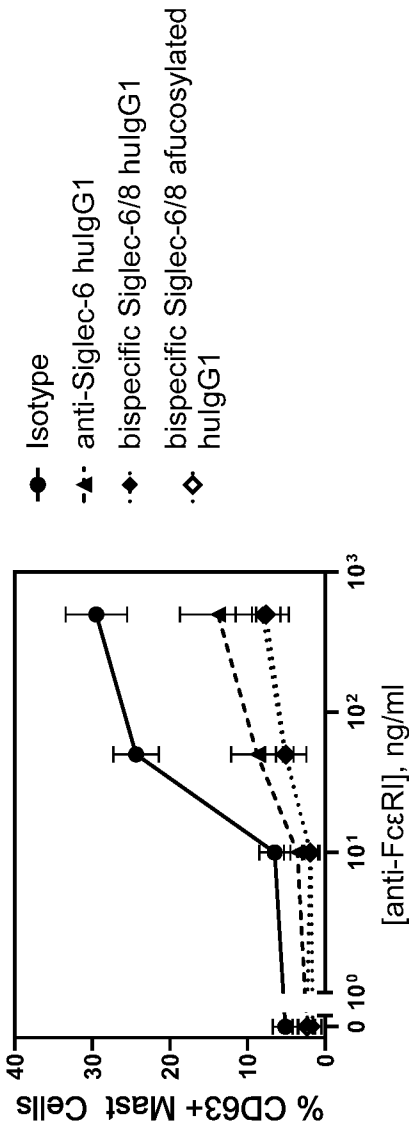


FIG. 6B

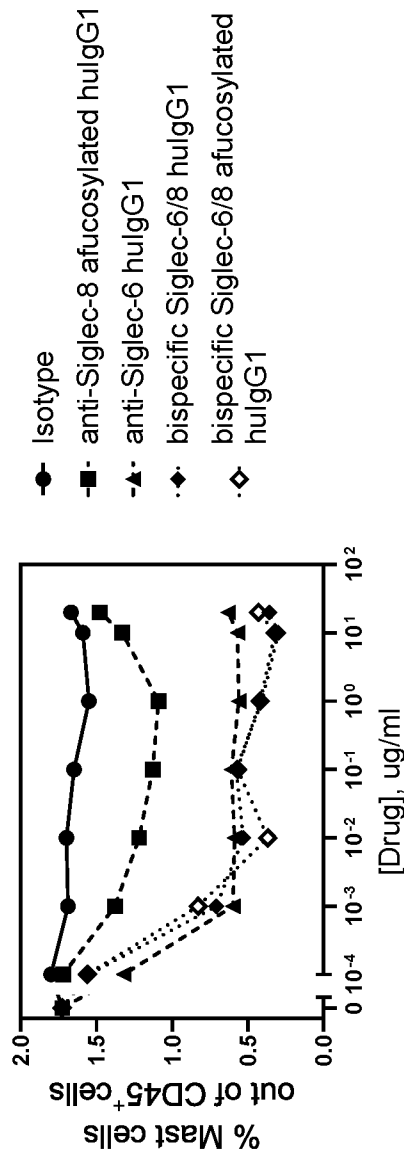


FIG. 7

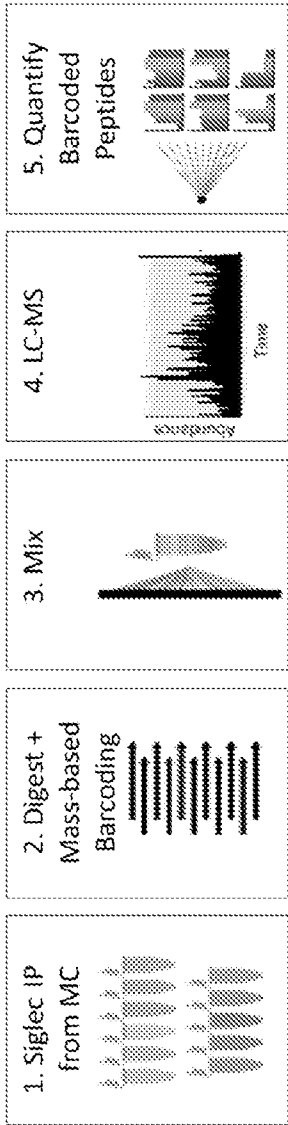


FIG. 8A

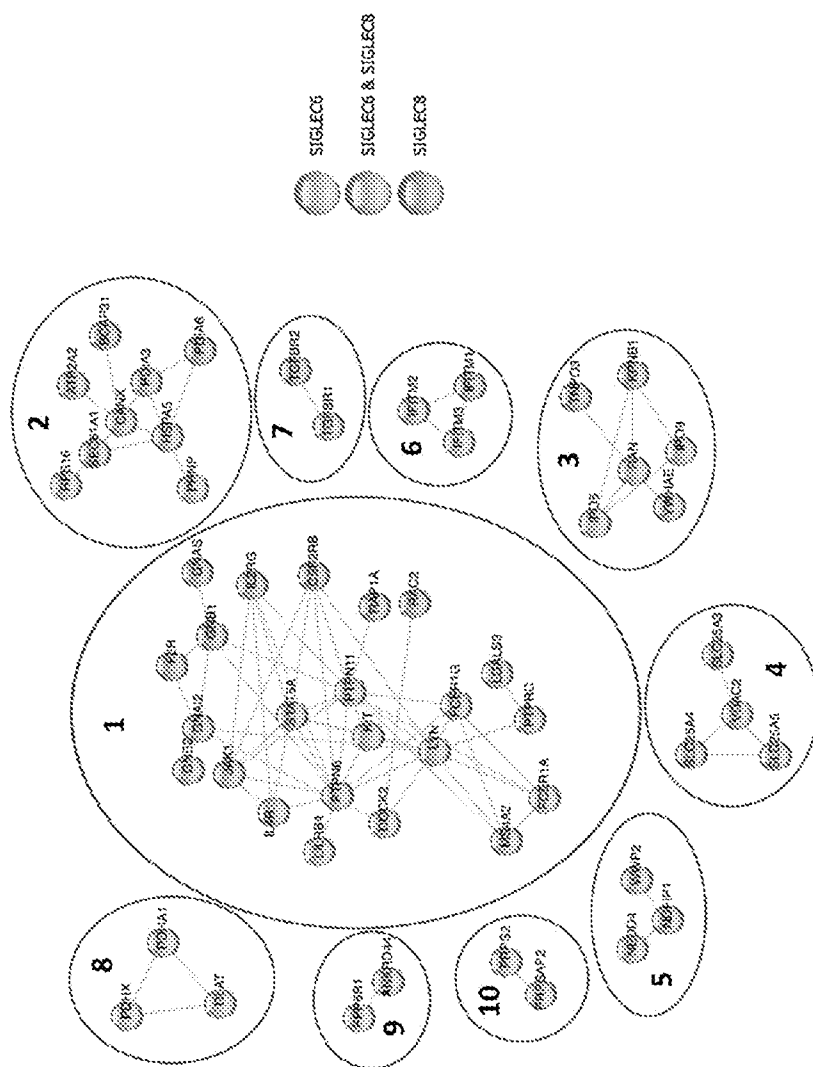


FIG. 8B

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/032993

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28 (2006.01) A61K 39/00 (2006.01) A61K 39/395 (2006.01) A61P 43/00 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

PATENW (C07K16/28, C07K2317/31, A61K39/395, Siglec-6, Siglec-8, Bispecific)

MEDLINE, CAPLUS, BIOSIS, EMBASE (Siglec-6, Siglec-8, Bispecific, J LEUNG, W KORVER, J SCHANIN, B YOUNGBLOOD)

Internal IP Australia databases (ALLAKOS INC., J LEUNG, W KORVER, J SCHANIN, B YOUNGBLOOD)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"D" document cited by the applicant in the international application	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 2 August 2024	Date of mailing of the international search report 02 August 2024
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Neal Dalton AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 2 6285 0740

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/US2024/032993
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2023/044390 A1 (ALLAKOS INC.) 23 March 2023 See abstract, 0006, 0174, Tables 2, 5, A, claims 9-23, 90-92, 107-109	1-130
Y	WO 2015/089117 A1 (ALLAKOS INC.) 18 June 2015 see Abstract, Tables 3, 4, 13, 0120, 0141, 0296, pages 108-114, claims 42-43, 78-85, 102-107	1-130
A	Schanin, J., et al. Discovery of an agonistic Siglec-6 antibody that inhibits and reduces human mast cells. Commun Biol 5, 1226 (2022). https://doi.org/10.1038/s42003-022-04207-w See whole document	1-130
A	Youngblood BA, et al. Discovery, Function, and Therapeutic Targeting of Siglec-8. Cells. 2020 Dec 24;10(1):19. doi: 10.3390/cells10010019. PMID: 33374255; PMCID: PMC7823959. see whole document	1-130
A	Robida PA, et al. Functional and Phenotypic Characterization of Siglec-6 on Human Mast Cells. Cells. 2022 Mar 28;11(7):1138. doi: 10.3390/cells11071138. PMID: 35406705; PMCID: PMC8997871. see whole document	1-130
A	O'Sullivan JA, et al. Eosinophil and mast cell Siglecs: From biology to drug target. J Leukoc Biol. 2020 Jul;108(1):73-81. doi: 10.1002/JLB.2MR0120-352RR. Epub 2020 Jan 22. PMID: 31965606; PMCID: PMC7531194. see whole document	1-130

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/US2024/032993	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2023/044390 A1	23 March 2023	WO 2023044390 A1	23 Mar 2023
		AU 2022347172 A1	04 Apr 2024
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/US2024/032993	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
		RS 59567 B1	31 Dec 2019
		SG 10201808835Q A	29 Nov 2018
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		US 2020054723 A1	20 Feb 2020
		US 2022331412 A1	20 Oct 2022
		ZA 201603435 B	26 Aug 2020
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2019)			