Clin Immunol., 2012, 130(3):563-71). There is currently a need for therapies that can control the activity of immune cells involved in inflammation, such as the activity of eosinophils and mast cells.

[0006] Previous studies have demonstrated that eosinophils undergo apoptosis when Siglec-8 is crosslinked with specific murine antibodies raised against the extracellular portion of Siglec-8 (Nutku et al., *Blood*, 2003, 336:918-24). These antibodies 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. However, there remains a need for developing humanized anti-Siglec-8 antibodies that recognize human Siglec-8 with high affinity and specificity. Discovery of such anti-Siglec-8 antibodies may allow for the development of treatment for diseases mediated by the activity of eosinophils and/or mast cells.

[0007] 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.

SUMMARY

[0008] Provided herein are anti-Siglec-8 antibodies (including humanized anti-Siglec-8 antibodies), compositions comprising thereof, and methods of using the same.

[0009] In one example, provided herein is a humanized antibody that specifically binds to a human Siglec-8, wherein the binding affinity and/or binding avidity of the humanized antibody to a human Siglec-8 are higher than the binding affinity and/or binding avidity of antibody 2E2 and/or antibody 2C4 to the human Siglec-8. In some examples, the human Siglec-8 is a dimer. In some examples, the human Siglec-8 comprises an extracellular domain human Siglec-8 fused to a Fc region of an immunoglobulin. In some examples, the Fc region is a human IgG1 Fc region. In some examples, the Fc region is a human IgG4 Fc region. In some examples, the human Siglec-8 comprises the amino acid sequence of SEQ ID NO:74.

[0010] In some examples, the humanized antibody comprises 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. In some examples, the 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 any of the examples herein, the antibody may comprise a heavy chain Fc region comprising a human IgG Fc region. In further examples, the human IgG Fc region comprises a human IgG1 or IgG4. In some examples, the human IgG1 comprises the amino acid sequence of SEQ ID NO:78. In some examples, the human IgG4 comprises the amino acid sequence of SEQ ID NO:79. In any of the examples herein, the antibody may comprise a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and/or a light chain comprising the amino acid sequence selected from SEQ ID NOs:76 or 77. In some examples, 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 any of the examples herein, the antibody may comprise a heavy chain comprising the amino acid sequence of SEQ ID NO:87; and/or a light chain comprising the amino acid sequence of SEQ ID NO:76. In some examples, the antibody has been engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In some examples, the antibody comprises two heavy chains and wherein at least one of the two or both heavy chains of the antibody is non-fucosylated.

[0011] In another example, provided herein is a humanized antibody that specifically binds to a human Siglec-8, wherein the antibody has a Tm of at least about 70ºC to at least about 72ºC in a thermal shift assay. In some examples, the antibody has a Tm at about 70ºC, at about 71ºC, or at about 72ºC in a thermal shift assay. In some examples, the antibody has the same or higher Tm as compared to a chimeric 2C4 antibody. In some examples, the antibody has the same or higher Tm 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.

[0012] In some examples, the antibody comprises 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. In some examples, the 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 any of the examples herein, the antibody may comprise a heavy chain Fc region comprising a human IgG Fc region. In further examples, the human IgG Fc region comprises a human IgG1 or IgG4. In some

examples, the human IgG1 comprises the amino acid sequence of SEQ ID NO:78. In some examples, the human IgG4 comprises the amino acid sequence of SEQ ID NO:79. In any of the examples herein, the antibody may comprise a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and/or a light chain comprising the amino acid sequence selected from SEQ ID NOs:76 or 77. In some examples, 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 any of the examples herein, the antibody may comprise a heavy chain comprising the amino acid sequence of SEQ ID NO:87; and/or a light chain comprising the amino acid sequence of SEQ ID NO:76. In some examples, the antibody has been engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In some examples, at least one or two of the heavy chains of the antibody is non-fucosylated.

[0013] In yet another example, provided herein is a humanized antibody that specifically binds to a human Siglec-8, wherein the antibody comprises 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:63 and 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 or 71. In some examples, the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:11-14; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:23-24. In some examples, 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 NO:63; and/or 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. In some examples, the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NO:6; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NO:16 or 21. In any of the examples herein, the antibody may comprise a heavy chain Fc region comprising a human IgG Fc region. In further examples, the human IgG Fc region comprises a human IgG1 or IgG4. In some examples, the human IgG1 comprises the amino acid sequence of SEQ ID NO:78. In some examples, the human IgG4 comprises the amino acid sequence of SEQ ID NO:79. In some

examples, 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 examples, the antibody has been engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In some examples, at least one or two of the heavy chains of the antibody is non-fucosylated.

[0014] In yet another example, provided herein is a humanized antibody that specifically binds to human Siglec-8, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:2-14; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16-24. In some examples, the antibody has been engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In some examples, at least one or two of the heavy chains of the antibody is non-fucosylated.

[0015] In yet another example, provided herein is a humanized antibody that specifically binds to human Siglec-8, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:2-10; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16-22. In some examples, the antibody has been engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In some examples, at least one or two of the heavy chains of the antibody is non-fucosylated.

[0016] In another example, provided herein is a humanized antibody that specifically binds to a human Siglec-8, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein (a) the heavy chain variable 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/or (b) the light chain variable region comprises: (1) an HC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:48-49; (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:64; (3) an HC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:51-53; (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:65; (5) an HC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:55-58; (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:66; and (7) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:60. In some examples, the antibody has been

engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In some examples, at least one or two of the heavy chains of the antibody is non-fucosylated.

[0017] In yet another example, provided herein is an isolated antibody that binds a human Siglec-8 and kills mast cells expressing Siglec-8 by ADCC activity. In some examples, the antibody kills mast cells expressing Siglec-8 *in vitro* (e.g., measured in a cell culture assay as described in Example 2). In some examples, the antibody depletes mast cells expressing Siglec-8 in a subject when a therapeutically effective amount is administered. In a further example, the antibody depletes at least about 20% (e.g. ,at least of the mast cells expressing Siglec-8 in a sample obtained from the subject as compared to a baseline level before treatment. In any of the examples herein, the sample can be a tissue sample or a biological fluid sample. In some examples, the tissue sample is one or more selected from the group consisting of: skin, lung, bone marrow, and nasal polyps. In some examples, the biological fluid sample is one or more selected from the group consisting of: blood, bronchoalveolar lavage, and nasal lavage. In any of the examples herein, the antibody can be engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In some examples, at least one or two of the heavy chains of the antibody is non-fucosylated. In further examples, the antibody may be produced in a cell line having a alpha1,6-fucosyltransferase (Fut8) knockout. In some further examples, the antibody may be produced in a cell line overexpressing β1,4-N-acetylglycosminyltransferase III (GnT-III). In further examples, the cell line additionally overexpresses Golgi μ-mannosidase II (ManII). In any of the examples herein, the antibody may comprise at least one amino acid substitution in the Fc region that improves ADCC activity. In any of the examples herein, the antibody may be a humanized antibody, a chimeric antibody or a human antibody. In some examples, the antibody is a human IgG1 antibody. In some examples, the antibody is a murine antibody. In any of the examples herein, the antibody may comprise 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. In a further example, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:106; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:109. In any of the examples herein, the antibody may comprise a heavy chain variable region comprising 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. In a further example, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:107; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:110. In any of the examples herein, the antibody may comprise a heavy chain variable region comprising a heavy chain variable region comprising 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. In a further example, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:108; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:111. In any of the examples herein, the antibody may comprise 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. In any of the examples herein, the antibody may comprise 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. In any of the examples herein, the subject can be a human.

[0018] In still another example, provided herein is an antibody that binds to a human Siglec-8 and a non-human primate Siglec-8. In any of the examples herein, the antibody may comprise a heavy chain variable region comprising 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. In a further example, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:107; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:110. In any of the examples herein, the antibody may comprise a heavy chain variable region comprising a heavy chain variable region comprising 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. In a further example, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:108; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:111. In any of the examples herein, the antibody may bind to an epitope in Domain 1 of human Siglec-8 (e.g., Domain 1 that comprises the amino acid sequence of SEQ ID NO:112). In any of the examples herein, the antibody may bind to an epitope in Domain 3 of human Siglec-8 (e.g., Domain 3 that comprises the amino acid sequence of SEQ ID NO:114). In any of the examples herein, the antibody may bind to an epitope in Domain 2 of human Siglec-8 (e.g., Domain 2 that comprises the amino acid sequence of SEQ ID NO:113). In any of the examples herein, the antibody may be a humanized antibody, a chimeric antibody or a human antibody. In some examples, the antibody is a murine antibody. In some examples, the antibody is an IgG1 or IgG4 antibody (*e.g.*, human IgG1 or IgG4).

[0019] In another example, provided herein is an anti-human Siglec 8 antibody that 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 examples, the antibody 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 examples, the antibody 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 examples herein, the antibody may comprise 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. In a further example, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:106; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:109. In some examples herein, the antibody may comprise a heavy chain variable region comprising 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. In a further example, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:107; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:110. In some examples herein, the antibody may comprise a heavy chain variable region comprising a heavy chain variable region comprising 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. In a further example, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:108; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:111.

[0020] In another example, provided herein is a humanized antibody that binds to a human Siglec-8, wherein the EC_{50} in depleting activated human eosinophils is less than the EC_{50} of antibody 2E2 or 2C4 to the human Siglec-8. In some examples, the EC_{50} of the humanized antibody is about 85% or less than the EC_{50} of antibody 2E2 or 2C4 to the human Siglec-8. In some examples, the EC_{50} of the humanized antibody is about 85%, about 80%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10% or about 5% or less than the EC_{50} of antibody 2E2 or 2C4 to the human Siglec-8. In any of the examples herein, the humanized antibody may comprise 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. In any of the examples herein, the humanized antibody may comprise 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 examples, the humanized antibody comprises 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. In any of the examples herein, the humanized antibody may comprise a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:2-14; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16-24. In any of the examples herein, the antibody may comprise 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. In any of the examples herein, the antibody may comprise a heavy chain variable region comprising 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. In any of the examples herein, the antibody may comprise a heavy chain variable region comprising a heavy chain variable region comprising 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. In any of the examples herein, the antibody may comprise a heavy chain Fc region comprising a human IgG Fc region. In further examples, the human IgG Fc region comprises a human IgG1 or IgG4. In some examples, the human IgG1 comprises the amino acid sequence of SEQ ID NO:78. In some examples, the human IgG4 comprises the amino acid sequence of SEQ ID NO:79. In any of the examples herein, the antibody may comprise a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and/or a light chain comprising the amino acid sequence selected from SEQ ID NOs:76 or 77. In some examples, 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 any of the examples herein, the antibody may comprise a heavy chain comprising the amino acid sequence of SEQ ID NO:87; and/or a light chain comprising the amino acid sequence of SEQ ID NO:76.

[0021] In another example, provided herein is a nucleic acid encoding any antibody described above and herein. In yet another example, provided herein is a vector comprising a nucleic acid described herein. In one example, the vector is an expression vector. In yet another example, provided herein is a host cell comprising a nucleic acid described herein. In some examples, the host cell expresses and produces the antibody.

[0022] In another example, provided herein is a method of producing an antibody comprising culturing a host cell comprising one or more nucleic acids encoding an antibody described herein under a condition that produces the antibody. In some examples, the method further comprises recovering the antibody produced by the host cell. Also provided herein is an anti-Siglec-8 antibody produced by the method. Also provided herein is an antigen-binding fragment of an anti-Siglec-8 antibody described herein.

[0023] In another example, provided herein is a pharmaceutical composition comprising an antibody described above and herein or an antigen-binding fragment thereof and a pharmaceutically acceptable carrier.

[0023a] In one aspect, the present disclosure refers to a composition comprising an antibody that specifically binds to human Siglec-8, wherein the antibody comprises a human IgG1 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.

[0024] In another example, provided herein is a composition comprising an antibody or fragment thereof that specifically binds to human Siglec-8, wherein the 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 in the composition contain a fucose residue. In some examples, substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some examples, the antibody is a humanized antibody, a chimeric antibody or a human antibody. In some examples, the antibody comprises 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. In some examples, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NOs:2-10; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NOs:16-22. In some examples, the antibody comprises 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. In some examples, the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:11-14; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:23-24. In some examples, the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:2-14; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16-24. In any of the examples herein, the composition may further comprise a pharmaceutically acceptable carrier. In any of the examples herein, the binding affinity and/or binding avidity of the antibody to a human Siglec-8 can be higher than the binding affinity and/or binding avidity of antibody 2E2 or 2C4 to the human Siglec-8. In any of the examples herein, the antibody may have a Tm of at least about 70ºC to at least about 72ºC in a thermal shift assay. In some examples, the antibody has a Tm of about 70ºC, about 71ºC, or about 72ºC. In some examples, the antibody has the same or higher Tm as compared to a chimeric 2C4 antibody. In some examples, the antibody has the same or higher Tm 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.

[0024a] In one aspect, the present disclosure refers to a kit comprising the composition as disclosed herein.

[0024b] In one aspect, the present disclosure refers to the composition as disclosed herein for use in therapy.

[0024c] In one aspect, the present disclosure refers to use of the composition as disclosed herein in the manufacture of a medicament for treating or preventing a disease mediated by cells expressing Siglec-8 in a subject.

[0025] In another example, provided herein is a method of treating or preventing a disease mediated by cells expressing Siglec-8 in a subject, the method comprising administering to the subject an effective amount of an antibody described herein or an antigen-binding fragment thereof or a composition described herein. In some examples, the disease is an eosinophil mediated-disease. In some examples, the disease is a mast cell mediated-disease. In some

examples, the disease is selected from the group consisting of asthma, allergic rhinitis, nasal polyposis, atopic dermatitis, chronic urticaria, mastocytosis, eosinophilic leukemia, and hypereosinophilic syndrome. In some examples, the disease is selected from the group consisting of pauci granulocytic asthma, acute or chronic airway hypersensitivity, eosinophilic esophagitis, 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, and allergic bronchopulmonary aspergillosis (ABPA). In some examples, the antibody inhibits one or more symptoms of an allergic reaction. In some examples, the allergic reaction is a Type I hypersensitivity reaction. In any of the examples herein, the subject may be suffering from asthma that is not adequately controlled by an inhaled corticosteroid, a short acting β2 agonist, a long acting β2 agonist, or a combination thereof.

[0026] In another example, provided herein is a method of depleting mast cells expressing Siglec-8 in a subject comprising administering to the subject an effective amount of an antibody that binds to human Siglec-8, wherein the antibody kills mast cells expressing Siglec-8 by ADCC activity. In some examples, the antibody kills mast cells expressing Siglec-8 *in vitro* (e.g., measured in a cell culture assay as described in Example 2). In some examples, the antibody depletes at least about 20% of the mast cells expressing Siglec-8 in a sample obtained from the subject as compared to a baseline level before treatment. In any of the examples herein, the sample can be a tissue sample or a biological fluid sample. In some examples, the tissue sample is one or more selected from the group consisting of: skin, lung, bone marrow, and nasal polyps. In some examples, the biological fluid sample is one or more selected from the group consisting of: blood, bronchoalveolar lavage, and nasal lavage. In any of the examples herein, the antibody can be engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In any of the examples herein, the antibody may comprise two heavy chains and wherein at least one of the two or both heavy chains of the antibody is non-fucosylated. In further examples, the antibody may be produced in a cell line having a alpha1,6-fucosyltransferase (Fut8) knockout. In some further examples, the antibody may be produced in a cell line overexpressing β1,4-N-acetylglycosminyltransferase III (GnT-III). In further examples, the cell line additionally overexpresses Golgi μ-mannosidase II (ManII). In any of the examples herein, the antibody may comprise at least one amino acid substitution in the Fc region that improves ADCC activity. In any of the examples herein, the antibody may be a humanized antibody, a chimeric antibody or a human antibody. In some examples, the antibody is a human IgG1 antibody. In any of the examples herein, the antibody may comprise 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. In a further example, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:106; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:109. In any of the examples herein, the antibody may comprise a heavy chain variable region comprising 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. In a further example, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:107; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:110. In any of the examples herein, the antibody may comprise a heavy chain variable region comprising a heavy chain variable region comprising 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. In a further example, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:108; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:111. In any of the examples herein, the antibody may comprise 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. In any of the examples herein, the antibody may comprise 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. In any of the examples herein, the subject has a disease mediated by cells expressing Siglec-8. In some examples, the disease is selected from the group consisting of asthma, allergic rhinitis, nasal polyposis, atopic dermatitis, chronic urticaria, mastocytosis, eosinophilic leukemia, and hypereosinophilic syndrome. In some examples, the disease is selected from the group consisting of pauci granulocytic asthma, acute or chronic airway hypersensitivity, eosinophilic esophagitis, 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, and allergic bronchopulmonary aspergillosis (ABPA). In some examples, the antibody inhibits one or more symptoms of an allergic reaction. In some examples, the allergic reaction is a Type I hypersensitivity reaction. In any of the examples herein, the subject may be suffering from asthma that is not adequately controlled by an inhaled corticosteroid, a short acting β2 agonist, a long acting β2 agonist, or a combination thereof. In any of the examples herein, the subject can be a human.

[0027] It is to be understood that one, some, or all of the properties of the various examples described herein may be combined to form other examples of the present disclosure. These and other examples of the disclosure will become apparent to one of skill in the art. These and other examples of the disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] **FIG. 1** is a sequence alignment showing the comparison of heavy chain sequences of humanized antibodies. Framework residues within 4Å of the CDRs in the molecular model of mouse 2E2 antibody are represented by a *; residues within 4Å of the CDRs and VCI residues that were different in the acceptor human framework are highlighted in ^, back-mutations of these residues in the acceptor human framework to mouse are indicated by @; Somatic mutations from human germline are indicated by °; if those residues were different from the AF471521 FW and mou2E2 (*i.e.*, mouse 2E2) or different from the AF471521 framework and identical to the mou2E2 (*i.e.*, mouse 2E2) the corresponding residues in the acceptor human framework were back-mutated to human germline and are indicated by +. Sequences highlighted

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising an antibody that specifically binds to human Siglec-8, wherein the antibody comprises a human IgG1 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.

2. The composition of claim 1, wherein substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue.

3. The composition of claim 1 or claim 2, wherein the antibody is a humanized or human antibody.

4. The composition of any one of claims 1-3, wherein the antibody comprises a heavy chain variable region and a light chain variable region, and 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 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.

5. The composition of any one of claims 1-3, wherein the antibody comprises a heavy chain variable region and a light chain variable region, and wherein the heavy chain variable region comprises an amino acid sequence selected from SEQ ID NOs:2-10, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:16-22.

6. The composition of any one of claims 1-3, wherein the antibody comprises a heavy chain variable region and a light chain variable region, and 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 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.

7. The composition of any one of claims 1-3, wherein the antibody comprises a heavy chain variable region and a light chain variable region, and wherein the heavy chain variable region comprises an amino acid sequence selected from SEQ ID NOs:11-14, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:23-24.

8. The composition of any one of claims 1-3, wherein the antibody comprises a heavy chain variable region and a light chain variable region, and wherein the heavy chain variable region comprises an amino acid sequence selected from SEQ ID NOs:2-14, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:16-24.

9. The composition of any one of claims 1-8, further comprising a pharmaceutically acceptable carrier.

10. The composition of any one of claims 1-9, wherein the binding affinity or avidity of the antibody to a human Siglec-8 is higher than the binding affinity or avidity of antibody 2E2 or 2C4 to the human Siglec-8.

11. The composition of any one of claims 1-10, wherein the antibody has a Tm of at least about 70ºC in a thermal shift assay.

12. The composition of claim 11, wherein the antibody has a Tm of at least about 70ºC to at least about 72ºC in a thermal shift assay.

13. A kit comprising the composition of any one of claims 1-12.

14. The composition of any one of claims 1-12 for use in therapy.

15. Use of the composition of any one of claims 1-12 in the manufacture of a medicament for treating or preventing a disease mediated by cells expressing Siglec-8 in a subject.

16. The use according to claim 15, wherein the disease is an eosinophil mediated-disease.

17. The use according to claim 15, wherein the disease is a mast cell mediated-disease.

18. The use according to claim 15, wherein the composition inhibits one or more symptoms of an allergic reaction.

19. The use according to claim 18, wherein the allergic reaction is a Type I hypersensitivity reaction.

20. The use according to claim 15, wherein the disease is selected from the group consisting of: asthma, allergic rhinitis, nasal polyposis, atopic dermatitis, chronic urticaria, mastocytosis, eosinophilic leukemia, and hypereosinophilic syndrome.

21. The use according to claim 15, wherein the disease is selected from the group consisting of: pauci granulocytic asthma, acute or chronic airway hypersensitivity, eosinophilic esophagitis, 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, and allergic bronchopulmonary aspergillosis (ABPA).

22. The use according to any one of claims 15-21, wherein the subject is suffering from asthma that is not adequately controlled by an inhaled corticosteroid, a short acting β2 agonist, a long acting β2 agonist, or a combination thereof.

23. The use according to any one of claims 15-22, wherein the subject is a human.